CROSSING OVER IN A CASE OF ATTACHED X CHROMOSOMES IN *DROSOPHILA MELANOGASTER*[1]

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INTRODUCTION

Among the primary non-disjunctional females obtained in a preliminary X-ray test (ANDERSON 1924) was one which gave only exceptional offspring. This line repeated the performance of a similar line studied by L. V. MORGAN (1922). In addition, several sex-linked genes were heterozygous, thus giving an opportunity to study crossing over between the two component chromosomes which had become attached to form a single V-shaped body.

ORIGIN AND CONSTITUTION

Scute apricot crossveinless tan forked ($s_c\ w^a\ c_v\ t\ f$) females were mated to broad echinus cut[2] garnet ($b_r\ e_c\ c_t\ g$) males. The female offspring were X-rayed soon after hatching, and mated to bar males. Some exceptional offspring were obtained, due to non-disjunction. One exceptional female was broad echinus in appearance, and, when mated to bar males, gave only

[1] Papers from the Department of Botany of the UNIVERSITY OF MICHIGAN, No. 227.

[2] The cut and garnet allelomorphs used were cut[s] and garnet[s].

broad echinus daughters and bar sons. These broad echinus daughters have repeated the same performance.

A number of female offspring obtained showed several characters in addition to broad and echinus. The additional characters obtained were crossveinless, cut, tan, garnet and forked. The genes for these five characters were expected to be present in heterozygous condition, so we may quite safely write the factorial constitution of the original broad echinus exception as $\frac{b_r\ e_c\ c_v\ t\ f}{b_r\ e_c\ \ c_t\ g}$. The character crossveinless proved so difficult to classify in the presence of broad, that it was neglected and the line permitted to become homozygous for the normal allelomorph. Since this line is homozygous throughout for the characters broad and echinus the genes for these characters will be omitted in the formulae and discussion to follow. The standard loci of the genes used are: cut 20.0, tan 27.5, garnet 44.4, and forked 56.5.

IDENTIFICATION OF PARENTAL CONSTITUTIONS

The absence of regular offspring makes the determination of the genetic constitution of the parent more difficult than in ordinary lines of Drosophila. The association of the recessive characters appearing among the daughters must be the main criterion. Only a few combinations of recessive characters have appeared in the progeny of any one female heterozygous for them. These combinations are assumed to represent the combinations of factors present in the two component parts of the double chromosome. Thus, a culture giving the combinations *t*, *t f*, *f*, *ct*, *ct g*, and *g* is interpreted as the offspring of a female of constitution $\frac{t\ f}{c_t\ g}$. Likewise, if a culture gives the combinations, *t*, c_t, *g*, *f*, c_t *g*, *g f* and c_t *g f*, its parental constitution is interpreted as $\frac{t}{c_t\ g\ f}$. As evidence of the correctness of these interpretations, it may be said that among the many cultures giving such combinations of recessives, only one individual of an unexpected combination has been observed. This individual is the *t f* female recorded in table 4.

Regular male and female offspring from the separation of the attached chromosomes also give evidence on the parental constitution. But their frequency is so low that they are only to be found in occasional cultures. The data obtained from the regular offspring are in accord with the parental constitutions assigned by the above method.

PHENOTYPIC PROGENY OF $\frac{t\ f}{c_t\ g}$ FEMALES

In general, only cultures from parents of constitution $\frac{t\ f}{c_t\ g}$ have been used for continuing the line. As a result, a considerable amount of data has been accumulated on the distribution of phenotypes in the progenies of females of this constitution. Table 1 gives the phenotypic data from those cultures in which the total number of female offspring is above 30. Smaller cultures are omitted, since in only a fraction of such cultures could the constitution be definitely identified. In order to insure the random character of these data an attempt was made to identify positively the parental constitution of all cultures giving 30 or more females. If any recessive character failed to appear, a mass culture was made up as an additional check.

TABLE 1

Progeny of $\frac{t\ f}{c_t\ g}$ *females* × *bar males.*

PEDIGREE NUMBER	MALES	+ ♀	*t* ♀	*tf* ♀	*f* ♀	c_t ♀	$c_t\ g$ ♀	*g* ♀	X-TRIPLOIDS	REGULAR
101	38	21	4	2	..	2	..	2	5	
102	25	18	7	1	..	7	1	..	..	
113	30	19	5	..	4	..	4	2	..	
121	54	22	5	2	3	1	3	1	2	
144	50	21	2	..	1	2	3	3	1	♂ $c_t\ g$
147	46	13	6	1	1	5	3	1	..	
163	49	26	3	3	1	2	1	2	..	
171	72	35	9	..	2	3	4	4	3	
172	88	48	8	2	1	5	4	5	17	
175	41	17	5	3	1	2	3	2	7	
181	116	63	13	2	2	9	5	7	13	
185	74	28	10	4	2	2	1	1	2	
186	69	40	3	1	..	2	1	1	..	♀
188	63	34	10	1	1	3	3	1	4	
189	84	53	5	2	..	1	3	1	..	
191	72	37	7	..	..	5	5	1	7	
195	71	45	5	2	1	5	4	2	3	♀ *t f/B*
197	45	19	6	2	..	3	2	1	3	
200	58	28	7	1	..	3	1	4	2	
201	76	26	9	5	..	6	8	2	12	
205	71	37	6	2	..	3	3	1	7	
208	50	24	3	..	..	6	4	1	7	
210	61	29	3	2	..	4	2	1	8	
211	25	21	6	2	..	3	..	1	..	♂ $c_t\ g$
212	60	43	5	2	1	5	1	1	5	

TABLE 1 (continued)

PEDIGREE NUMBER	MALES	+♀	*t*♀	*tf*♀	*f*♀	c_t♀	$c_t g$♀	*g*♀	X-TRIPLOIDS	REGULAR
220	55	28	7	1	..	4	1	..	..	
222	38	25	6	2	1	3	3	1	..	
224	47	14	6	2	1	4	4	..	1	
225	37	20	4	4	1	2	4	1	..	
228	16	21	3	1	2	3	..	2	3	
237	31	21	3	..	1	3	1	1	..	
239	60	25	6	1	1	4	6	2	1	
240	75	45	6	4	2	6	6	4	5	
241	68	46	6	..	1	6	2	..	..	
242	41	14	5	3	3	3	2	4	..	
244	62	25	6	1	1	7	8	2	4	
245	51	27	7	1	..	7	..	1	1	
246	64	22	10	3	2	5	5	..	2	
247	47	40	4	1	1	7	2	..	..	
257	48	42	2	..	..	10	4	..	8	♂ *tf*
258	84	33	8	1	2	7	7	1	10	
262	30	22	3	3	1	2	4	1	..	
263	45	31	3	3	..	3	4	..	..	
265	44	27	11	4	..	4	8	2	1	
267	57	22	7	5	..	4	2	2	3	
268	29	27	6	..	1	4	5	3	..	
271	46	31	9	2	..	6	1	3	..	
272	61	46	6	5	4	8	10	4	..	
273	45	26	3	3	1	5	3	4	..	
274	59	60	7	2	2	6	3	2	..	
275	46	28	8	..	..	4	2	1	..	
276	78	46	17	3	1	7	7	3	4	♂ c_t *gf*
277	72	49	9	2	1	6	4	4	2	
278	62	47	11	..	1	5	2	4	..	
279	33	22	2	1	..	4	2	2	..	
280	42	23	6	2	2	4	3	1	..	
287	22	24	3	..	..	3	3	3	..	
291	82	53	8	2	1	7	1	..	2	
293	30	25	7	1	..	3	..	3	..	
294	42	22	2	3	..	4	3	..	..	
296	51	28	5	3	..	8	1	..	2	
298	54	44	5	2	1	8	4	..	2	
Total	3342	1918	379	113	56	275	196	109	159	6
Percent of ♀♀		63.0	12.4	3.7	1.8	9.0	6.4	3.6		

Individuals which show one only of the recessive characters which were heterozygous in the parent, are due to a portion of one parental chromosome becoming homozygous, while another portion remains heterozygous. The two chromosomes are therefore neither identical nor complementary.

The fact that more than one-fourth of all the female offspring are of that type is ample proof that crossing over occurs after each chromosome has become divided, that is, in the four-strand stage. An hypothesis of crossing over in the four-strand stage was used by BRIDGES (1916) to account for non-disjunctional females obtained, in which one or more of the heterozygous factors of the female parent had become homozygous (the so-called "equational" exceptions). The hypothesis of crossing over in the four-strand stage has also been substantiated by data obtained on crossing over in triploids (BRIDGES and ANDERSON 1925).

PHENOTYPIC PROGENY OF OTHER TYPES OF BROAD ECHINUS FEMALES

Tables 2, 3 and 4 give the phenotypic progeny of broad echinus females heterozygous for c_t, t, g and f, but with these genes associated differently from those of table 1. As in table 1, cultures giving less than 30 females are omitted. The single $t\,f$ female in table 4 was tested and proved to be of constitution $\frac{t\ \ f}{t\ g\ f}$. Both chromosomes were therefore crossovers, but in different regions.

TABLE 2

Progeny of $\frac{c_t t\ \ f}{g}$ *females.*

PEDIGREE NUMBER	MALES	+	c_t	$c_t\,t$	$c_t\,t\,f$	$t\,f$	f	t	g	X-TRIPLOIDS	REGULAR
184	77	36	1	5	1	..	..	..	5	1	
209	36	31	2	9	1	..	1	1	1	5	♀*f*/*B*
243	71	35	4	7	1	1	1	2	8	4	
264	79	53	1	6	4	..	..	..	1	12	
297	59	49	2	7	1	..	..	1	4	..	
Total ♀♀	322	204	10	34	8	1	2	4	19	22	1

TABLE 3

Progeny of $\frac{t\ g}{c_t\ \ f}$ *females.*

PEDIGREE NUMBER	MALES	+	t	$t\,g$	g	c_t	$c_t\,f$	f	X-TRIPLOIDS
110	45	29	4	3	..	4	4	..	..
156	46	28	3	4	..	3	3	..	..
174	73	32	6	4	3	4	..	1	3
198	65	43	2	1	..	7	2	..	1
266	55	36	7	5	1	4	4	..	6
281	38	32	4	2	4	7	..	1	1
300	41	25	3	4	..	4	3	1	..
Total ♀♀	363	225	29	23	8	33	16	3	11

TABLE 4

Progeny of $\frac{c_t\ g\ f}{t}$ *females.*

PEDIGREE NUMBER	MALES	+	t	c_t	$c_t\ g$	$c_t\ g\ f$	$g\ f$	f	g	$t\ f$	X-TRIPLOIDS
103	47	25	5	3	3	..	1	2	..	..	8
119	51	45	6	5	1	..	1	..	..	..	2
122	62	29	6	4	5	1	..	..	1	..	1
123	47	23	5	2	2	..	1	..	..	..	..
124	50	24	6	2	..	1	..	..	..	..	..
125	28	20	6	9	1	2	..	..	..	..	..
130	31	21	6	1	..	..	1	1	1	..	..
137	63	19	5	4	3	..	2	..	..	..	2
146	40	18	8	3	..	1	2	..	..	..	..
182	81	50	14	1	3	1	1	..	1	..	5
190	54	46	10	3	2	1	..	1	1	..	4
206	73	32	4	2	3	1	..	..	..	..	9
207	56	29	3	4	1	1	..	..	2	..	8
255	36	36	15	3	4	2	1	..	..	1	..
283	56	40	7	6	3	1	..	..	..	..	1
Total ♀ ♀	775	457	106	52	31	12	10	4	6	1	40

FREQUENCY OF APPEARANCE OF RECESSIVE CHARACTERS

From the data of tables 1 to 4 the total frequency of appearance of each recessive character may be calculated. These frequencies are summarized in table 5. Cut and tan appeared most frequently, 15.5 and 16.1 percent, respectively. Garnet appeared in 9.5 percent, while forked, the least frequent, appeared in only 5.2 percent of the females. The decreasing frequencies toward the forked end of the chromosome indicate that the point of attachment of the two chromosomes is at this end. The appearance of forked is due to crossing over between forked and the point of attachment.

TABLE 5

The frequency of appearance of the recessive characters, cut, tan, garnet and forked, in progenies of broad echinus females heterozygous for these characters.

	TABLE 1	TABLE 2	TABLE 3	TABLE 4	TOTAL	PERCENT OF TOTAL ♀ ♀
Cut	471	52	49	100	672	15.5
Tan	492	47	52	107	698	16.1
Garnet	305	19	31	59	414	9.5
Forked	169	11	19	27	226	5.2
Total females	3046	282	337	679	4344	

If the four strands present at the time of crossing over were assorted at random, any one recessive character heterozygous in the parent should appear in one-sixth of the female offspring. The percentages for cut and tan are only slightly below 15.7 percent, but the difference is probably significant. The deviation for cut is nearly 3.2 times the probable error, corresponding to a probability of 3.1 per hundred. The odds against such a distribution from chance alone are 30 to 1. Such a deficit might well be due to slightly lower viability of the homozygous forms, or to assortment not being wholly random. Investigation under way by Doctor A. H. STURTEVANT on a somewhat similar case of attached X chromosomes heterozygous for the entire length of the chromosome should throw more light on this problem.

GENOTYPIC PROGENY OF $\frac{t\ f}{c_t\ g}$ FEMALES

An attempt was made to identify the genotypic constitutions of daughters from $\frac{t\ f}{c_t\ g}$ mothers. A total of 188 identifications were made. These are given in table 6. These identifications are not evenly distributed among the different phenotypes, so in calculating the percentages

TABLE 6

Genotypic constitution of female progeny of $\frac{t\ f}{c_t\ g}$ *females.*

GENOTYPE	PHENOTYPE	NUMBER	PROPORTIONAL PERCENTAGE	SCHEMATIC REPRESENTATION	CROSSING OVER
$\frac{t\ f}{c_t\ g}$	+	76	43.9	$\frac{a\ a\ a\ a}{b\ b\ b\ b}$	0-0
$\frac{c_t\ t\ f}{g}$	+	7	4.0	$\frac{b\ a\ a\ a}{a\ b\ b\ b}$	1-1
$\frac{t\ g}{c_t\ f}$	+	9	5.2	$\frac{a\ a\ b\ b}{b\ b\ a\ a}$	2-2
$\frac{t}{c_t\ g\ f}$	+	9	5.2	$\frac{a\ a\ a\ b}{b\ b\ b\ a}$	3-3
$\frac{g\ f}{c_t\ t}$	+	1	0.6	$\frac{a\ b\ b\ a}{b\ a\ a\ b}$	1,3-1,3
$\frac{t\ f}{g}$	+	3	1.7	$\frac{a\ a\ a\ a}{a\ b\ b\ b}$	0-1
$\frac{c_t\ g}{c_t\ t\ f}$	c_t	3	1.8	$\frac{b\ b\ b\ b}{b\ a\ a\ a}$	0-1
$\frac{t\ f}{t\ g}$	t	13	4.8	$\frac{a\ a\ a\ a}{a\ a\ b\ b}$	0-2

TABLE 6 (continued)

GENOTYPE	PHENOTYPE	NUMBER	PROPORTIONAL PERCENTAGE	SCHEMATIC REPRESENTATION	CROSSING OVER
$\frac{c_t\ g}{c_t\ f}$	c_t	12	7.2	$\frac{b\ b\ b\ b}{b\ b\ a\ a}$	0–2
$\frac{t\ f}{t}$	t	19	7.0	$\frac{a\ a\ a\ a}{a\ a\ a\ b}$	0–3
$\frac{c_t\ g}{c_t\ g\ f}$	$c_t\ g$	6	4.3	$\frac{b\ b\ b\ b}{b\ b\ b\ a}$	0–3
$\frac{c_t\ t\ f}{t\ g}$	t	2	0.7	$\frac{b\ a\ a\ a}{a\ a\ b\ b}$	1–2
$\frac{g}{c_t\ g\ f}$	g	1	0.4	$\frac{a\ b\ b\ b}{b\ b\ b\ a}$	1–3
$\frac{c_t\ g}{f}$	+	1	0.6	$\frac{b\ b\ b\ b}{a\ b\ a\ a}$	0–1,2
$\frac{c_t\ g}{g\ f}$	g	1	0.4	$\frac{b\ b\ b\ b}{a\ b\ b\ a}$	0–1,3
$\frac{c_t\ g}{t\ g\ f}$	g	1	0.4	$\frac{b\ b\ b\ b}{a\ a\ b\ a}$	0–2,3
$\frac{t\ g}{g\ f}$	g	1	0.4	$\frac{a\ a\ b\ b}{a\ b\ b\ a}$	2–1 3
$\frac{t}{g\ f}$	+	1	0.6	$\frac{a\ a\ a\ b}{a\ b\ b\ a}$	3–1,3
$\frac{t\ f}{t\ f}$	$t\ f$	4	2.5	$\frac{a\ a\ a\ a}{a\ a\ a\ a}$	0–0
$\frac{c_t\ g}{c_t\ g}$	$c_t\ g$	3	2.1	$\frac{b\ b\ b\ b}{b\ b\ b\ b}$	0–0
$\frac{t\ f}{c_t\ t\ f}$	$t\ f$	2	1.2	$\frac{a\ a\ a\ a}{b\ a\ a\ a}$	0–1
$\frac{c_t\ g}{g}$	g	4	1.6	$\frac{b\ b\ b\ b}{a\ b\ b\ b}$	0–1
$\frac{t\ f}{c_t\ f}$	f	5	1.5	$\frac{a\ a\ a\ a}{b\ b\ a\ a}$	0–2
$\frac{c_t\ g}{t\ g}$	g	1	0.4	$\frac{b\ b\ b\ b}{a\ a\ b\ b}$	0–2
$\frac{t\ f}{c_t\ g\ f}$	f	1	0.3	$\frac{a\ a\ a\ a}{b\ b\ b\ a}$	0–3
$\frac{c_t\ g}{t}$	+	2	1.2	$\frac{b\ b\ b\ b}{a\ a\ a\ b}$	0–3

this must be taken into consideration. The percentages given are calculated as proportional parts of the percentages of phenotypes given in table 1. For convenience in analysis there is included a schematic repre-

sentation of the genotypic constitutions using "a" to represent any gene from the *t f* parental chromosome and "b" to represent any gene from the *c₁ g* parental chromosome.

CROSSING OVER

The 188 individuals identified, each having two chromosomes from the female parent, give 376 chromosomes from which to calculate crossing over. The percentages of crossing over calculated from the percentage frequencies of table 6 are given in table 7. For comparison, the standard expectations for crossing over are given and also the percentages of crossing over found by BRIDGES when using the parental stocks in other experiments. The deviations from expectations are no greater than might be expected from random sampling alone. The coincidence values for double crossing over are: 0.2 for regions 1 and 2; 1.0 for regions 1 and 3; and 0.1 for regions 2 and 3. Considering the small numbers involved, these are quite close to the standard expectations. Thus the data on percentages of crossing over and on coincidence show that crossing over proceeds normally and regularly even though the chromosomes are attached.

TABLE 7

Summary of crossing-over data from table 6.

REGION	FREQUENCY	TOTAL FREQUENCY	PERCENT OF CROSSING OVER	STANDARD	DATA FROM BRIDGES
0	131.4				
1	15.4	18.6	9.3	7.5	9.2
2	25.4	26.4	13.2	16.9	17.2
3	24.2	27.2	13.6	12.1	12.6
1,2	0.6				
1,3	2.6				
2,3	0.4				

ASSOCIATION OF CHROMOSOMES

A summary of the data on association of component chromosomes, from table 6, gives a little information on the mechanism of crossing over and of assortment of the resulting strands. When only a single region is considered, with the two pairs of genes which limit the region, the data may be summarized in relatively simple form. The possible types of association are:

(1) Both chromosomes non-crossovers and complementary.
(2) Both chromosomes non-crossovers and identical.
(3) One chromosome a crossover, the other a non-crossover.
(4) Both chromosomes crossovers and complementary.
(5) Both chromosomes crossovers and identical.

Type 3 may be divided into two subtypes, one with the left locus homozygous $\frac{a\,b}{a\,a}$, the other with the right locus homozygous $\frac{b\,a}{a\,a}$. With this classification as a basis, the data have been summarized and are given in table 8.

TABLE 8

Summary of data from table 6 on association of chromosomes in single regions of the attached chromosomes.

TYPE OF ASSOCIATION	FORMULA	1	2	3
1. Complementary non-crossovers	$\frac{aa}{bb}$	58.1	59.3	69.9
2. Identical non-crossovers	$\frac{aa}{aa}$	27.9	19.5	9.3
3. Crossover and non-crossover (a)	$\frac{ab}{aa}$	4.5	13.3	12.9
3. Crossover and non-crossover (b)	$\frac{ba}{aa}$	4.9	2.7	1.5
4. Complementary crossovers	$\frac{ab}{ba}$	4.6	5.2	6.4
5. Identical crossovers	$\frac{ab}{ab}$	0.0	0.0	0.0

The most significant feature of this summary is the entire absence of the type in which the two components are identical crossovers. This shows that only two of the four strands are crossovers at any one level. If all four strands ever show crossing over at any one level, such cases must be rare. The data from tables 1 to 4 likewise give no indication of such cases. The interpretation that crossing over involves only two strands at any one level has also been derived from the study of crossing over in triploids (BRIDGES and ANDERSON 1925). On this interpretation the products of crossing over in a given region will be two complementary crossovers and two complementary non-crossovers. Random assortment of these four strands would give X-bearing eggs of the following types:

16.7 percent complementary non-crossovers
66.7 percent one crossover and one non-crossover
16.7 percent complementary crossovers

Thus the numerical proportion between types 3 and 4 of table 9 becomes one index of the randomness of assortment of strands at the reduction division. When the totals for the three regions are considered, the type with both chromosomes crossovers is above the expectation for random

assortment. The observed ratio is 16.2 to 39.8; the random expectation, 11.2 to 44.8. The deviation is 2.5 times the probable error and the consistency in the three separate regions is further evidence that the difference is significant. Thus, the data indicate that there is a disproportionate tendency for any crossover chromosome to be associated with its complement. Since randomness of assortment is disturbed by attachment of the chromosomes at the right, or forked end, it becomes more instructive to consider assortment beginning at the point of attachment. Here no recombination can have occurred, so only the parental combination $\frac{a}{b}$ is possible. This must continue (toward the left) to the point of the rightmost crossover, where the continuity of the strands is first broken. The rightmost observed crossovers are tabulated in table 9. For region 4, not included in the table, the frequency for class 3 (crossover and non-crossover) is 10.8, but class 4 (complementary crossovers) is not detectable. Classes 2, $\left(\frac{a\,a}{a\,a}\right)$, 3b, $\left(\frac{b\,a}{a\,a}\right)$, and 5, $\left(\frac{a\,b}{a\,b}\right)$, cannot occur except when there is an intervening crossover between the region under consideration and the point of attachment. Therefore, they do not appear in the table.

TABLE 9

Summary of rightmost crossovers from table 6.

TYPE OF ASSOCIATION	FORMULA	1	2	3	TOTAL
(1) Complementary non-crossovers.....	$\frac{aa}{bb}$	43.9	51.4	69.9	
(3a) Crossover and non-crossover.....	$\frac{ab}{aa}$	3.5	13.3	12.9	29.7
(4) Complementary crossovers.....	$\frac{ab}{ba}$	4.0	5.2	6.4	15.6

Crossing over has been shown (page 412) to involve only two strands, the other two strands remaining non-crossovers at that level. If crossing over is random between strands, that is, if a given strand is equally likely to cross over with any one of the other strands, the ratio of class 3 $\left(\frac{ab}{aa}\right)$ to class 4 $\left(\frac{ab}{ba}\right)$ should be 2 to 1. The totals for the three regions are 29.7 and 15.6, very close indeed to the random expectation. The discrepancies in regions 1 and 2 are not in serial order and may well be due to errors of sampling. The data, then, support the hypothesis (BRIDGES and ANDER-

SON 1925, and the preliminary account in MORGAN, STURTEVANT and BRIDGES 1924), that assortment is determined at the right or spindle-fiber end, that crossing over occurs at the four-strand stage, but only two of the four strands cross over at any one level, and that crossing over occurs freely, if not at random, between any two strands. On this hypothesis, randomness of assortment of crossovers should be approached only gradually in progressing from right to left along the chromosome map.

The ratio between the two types of non-crossover combinations varies in the different regions due to proximity to the point of attachment. The expected ratio on random assortment is easily calculated for regions sufficiently distant from the point of attachment that the percentage of phenotypic appearance of recessive characters is not reduced, which condition is approximated only for region 1. The class of complementary non-crossovers includes some derived from cases in which crossing over has taken place between the other two strands. These should equal in number the complementary crossovers (type 4 of table 8). When these are subtracted the difference should equal twice the frequency of the identical non-crossover class if assortment is at random. For region 1 the difference between the complementary non-crossover and complementary crossover frequencies is 53.5. The identical non-crossover frequency is 27.8. The expectation for random assortment is 54.2 to 27.1. The deviation is very slight, much less than the probable error, which shows that assortment of non-crossovers is at random for this section.

THE POINT OF ATTACHMENT OF THE X CHROMOSOMES

If the characteristics of crossing over and assortment which prevail throughout the region from cut to forked are continued beyond forked to the point of attachment, this distance can be calculated. The frequency value for crossovers between forked and the point of attachment will be the frequencies of homozygosis of forked and of the normal allelomorph of forked plus twice the frequency of combinations of complementary crossovers. The latter cannot be detected, but if assortment is the same in this region as in the others under observation, they should be half as frequent as the crossover-non-crossover combinations which lead to homozygosis of *f* or its allelomorph. The percent of crossing over between forked and the point of attachment then becomes equal to the percent of homozygosis of *f* and its allelomorph. This gives a value of 10.8 from the data in table 6. From the phenotypic data in table 5 a corresponding value of 10.4 is obtained. These values would place the point of attachment in the neighborhood of "bobbed," the rightmost known gene, which had

previously been mapped at 70± by BRIDGES and STURTEVANT. Presumably the point of attachment is at the very end of the chromosome and the locus of bobbed very near there.

DETACHMENTS

The attached X chromosomes characteristic of this line, occasionally become detached. These detachments give rise to regular offspring. Four regular males and 3 regular females are recorded in tables 1 to 4. In the same tables are recorded 4802 bar males and 4344 broad echinus females. The proportion of regular offspring is thus about 1 per 1300. Since all the eggs resulting from detachment should be fertile, whereas only one-half of the XX and Y eggs give viable zygotes, the frequency of detachment

TABLE 10

Constitution and origin of regular offspring from detachment of attached X chromosomes.

SEX	CONSTITUTION OF CHROMOSOME FROM FEMALE PARENT	CONSTITUTION OF FEMALE PARENT	CROSSING-OVER REGION	REGIONS UNDER OBSERVATION
♂	$t\ f$	$\frac{t\ f}{c_t\ g}$	0	1, 2, 3
♀	$t\ f$	$\frac{t\ f}{c_t\ g}$	0	1, 2, 3
♂	$c_t\ g$	$\frac{t\ f}{c_t\ g}$	0	1, 2, 3
♂	$c_t\ g$	$\frac{t\ f}{c_t\ g}$	0	1, 2, 3
♂	$c_t\ g\ f$	$\frac{t\ f}{c_t\ g}$	3	1, 2, 3
♀	f	$\frac{c_t\ t\ f}{g}$	2	1, 2, 3
♀	$g\ f$	$\frac{t}{g\ f}$	0	2, 3
♀	$t\ g$	$\frac{t\ f}{t\ g}$	0	3
♂	t	$\frac{t\ f}{t}$	0	0
♂	$t\ g$	$\frac{t\ f}{t\ g}$	0	3
♂	$c_t\ g$	$\frac{c_t\ g\ f}{c_t\ g}$	0	0
♂	t	$\frac{t\ f}{t\ g}$	3	3

is only about 1 per 2600. The fact that no culture has given more than one regular male or female indicates that the detachments occur at or near the maturation divisions.

The X constitutions of six of the above regular males and females have been identified. In addition, six others of known parentage have been identified. A tabulation of the constitutions of parents and regular offspring is given in table 10. Three of the twelve are crossovers in regions under control. For region 1 there were no crossovers out of six cases. For region 2 there was one crossover out of seven cases. For region 3 there were two crossovers out of ten cases. It is evident that detachment of attached chromosomes is not strongly correlated with the presence or absence of crossing over. It is probable that the distribution of crossing over in chromosomes which become detached is similar to that in chromosomes which remain attached.

SUMMARY

From an X-rayed F_1 female of a cross between scute apricot crossveinless tan forked ($s_c\ w^a\ c_v\ t\ f$) by broad echinus cut garnet ($b_r\ e_c\ c_t\ g$), one non-disjunctional daughter was obtained which gave only exceptional offspring. In appearance it was broad echinus. Its X constitution was $\frac{b_r\ e_c\ c_v\ t\ f}{b_r\ e_c\ \ c_t\ g}$, the two X chromosomes being attached at the forked end. When such a female is mated to a bar male, only broad echinus females and bar males are produced.

Among the broad echinus daughters are some which show one or more of the recessive characters which were heterozygous in the parent. In general, only the genes present in one of the two attached chromosomes become homozygous in any one daughter.

Data have been collected on the frequency of appearance of the recessive characters cut, tan, garnet and forked in the female progeny of heterozygous mothers of known constitution. The frequencies are: cut 15.5, tan 16.1, garnet 9.5, forked 5.2.

The data presented are evidence that crossing over occurs at a four-strand stage. If the four strands were assorted at random, each character should appear in one-sixth of the daughters. The percentages for cut and tan approach, but do not quite equal, the expectation for random assortment. The decreasing percentages from tan to forked indicate that the chromosomes are attached at the forked end.

A study of the genotypic constitutions of 188 broad echinus daughters shows that crossing over has taken place about as expected for these factors in ordinary lines of Drosophila.

Evidence is presented showing that crossing over takes place between only two of the four strands at any one level.

When a single crossover region only is considered, a crossover chromosome may be associated with its complement or with a non-crossover chromosome. When only the crossovers nearest to the point of attachment are considered, crossing over appears to be random, association of strands being determined by their attachment. These data support the hypothesis that assortment of chromosomes is determined at the right or spindle-fiber end, that crossing over occurs at the four-strand stage but only two strands cross over at any one level, and that crossing over occurs freely between any two strands.

The point of attachment calculated from the crossing-over data and the percentage of homozygosis of forked, should be about 10.6 units beyond forked.

Occasionally, the attached chromosomes become detached, giving rise to regular male or female offspring. The frequency of regular offspring is about 1 per 1300. These regular offspring may be crossovers or non-crossovers. The percent of crossing over is probably similar to that shown by the exceptional daughters.

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CROSSING OVER IN THE X CHROMOSOMES OF TRIPLOID FEMALES OF *DROSOPHILA MELANOGASTER*[1]

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INTRODUCTION

An investigation of crossing over in triploid females of Drosophila was undertaken, partly from an interest in the problem of crossing over where three instead of two homologous chromosomes are involved, and partly in hope that it would throw light on the mechanism of crossing over under the ordinary diploid condition. The advantage of using triploids is that over forty percent of their offspring come from eggs in which two homologous chromosomes, instead of one, have been derived from a single process of maturation. The constitution of the other homologues derived from that same maturation, but lost to the polar body, can only be deduced from the characteristics of the two that can be directly observed. Such an opportunity to analyse the products of a reduction division had previously been restricted to the few sporadic cases of so-called "equational

[1] Papers from the Department of Botany of the UNIVERSITY OF MICHIGAN, No. 226.

non-disjunction" (BRIDGES 1916). Subsequently, two other means have arisen. One is the study of crossing over in cases in which two X chromosomes are permanently attached to one another (ANDERSON 1925, L. V. MORGAN 1925, A. H. STURTEVANT unpublished). The other is by artificially increasing the frequency of "equational exceptions" through use of X rays (unpublished results of ANDERSON). These four means of analysis give results that, so far as can be judged at present, are in substantial agreement. In this paper we intend to present only the facts immediately derived from the study of the *3n* female, leaving the more general considerations for treatment when the data from all four methods of analysis are available for comparison. The present study is restricted to the X chromosomes, because of technical difficulties in the case of the autosomes. For previous information on *3n* females, see BRIDGES 1921, 1922. A preliminary account of the present experiments and findings has been included in the Year Book of the CARNEGIE INSTITUTION OF WASHINGTON 1923, p. 286.

PLAN

The general plan of our test was to synthesize triploids of known constitution with a sufficient number of factor differences so arranged that any considerable portion of any X chromosome could be identified in the progeny. The progeny to be studied were the exceptional diploid females, since each of them carried two X chromosomes from their triploid mother. Previous data (BRIDGES) had shown that these form about 41 percent of the total offspring of triploid mothers. The total distribution is shown

TABLE 1

Offspring of 3n ♀ × bar ♂. (BRIDGES.)

EGG	SPERM	ZYGOTE	TOTALS	PERCENT
2X2A*	XA	*3n* female	105	3.9
	YA	Intersex, exceptional	104	3.8
1X1A	XA	*2n* ♀, regular	154	5.7
	YA	Male, regular	162	6.0
2X1A	XA	Superfemale	36	1.3
	YA	*2n* ♀, exceptional	1116	41.1
1X2A	XA	Intersex, regular	898	33.1
	YA	Supermale	142	5.2

* The symbol A is used to designate a set composed of one representative of each kind of autosome.

in table 1. The productivity of the triploid is quite low, the total progeny averaging about 60 individuals, the number of exceptional females averaging about 25.

REQUIRED CONSTITUTION OF THE TRIPLOID

In order to identify crossovers, it was necessary to have each of the three X chromosomes of the triploid differing by known genes at intervals. Five points on the map (I, II, III, IV and V, figure 1) were selected, at or near each of which two different genes were located. For example, with regard to the leftmost of these points (I), one chromosome may be marked by the recessive mutant yellow, the second by the normal allelomorph of yellow, and the third by scute, a recessive that ordinarily shows no crossing over with yellow. The following chromosome map shows graph-

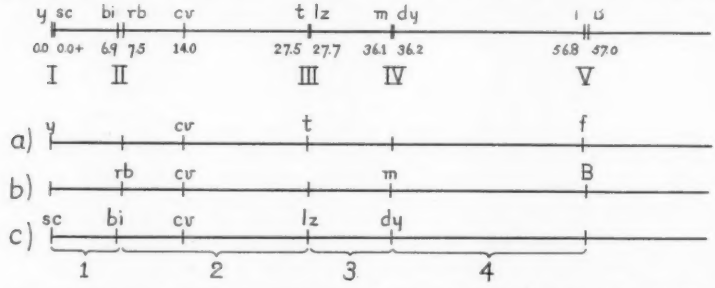

FIGURE 1.—Maps of the X chromosome showing standard loci of genes used in studies on crossing over in the triploid and a graphic representation of the constitution of the triploid females.

ically the location of the selected genes (figure 1, upper line). The distance between yellow and scute is 0.0 units, between bifid and ruby is 0.6 units, between tan and lozenge 0.2 units, between miniature and dusky 0.1 units, between forked and bar 0.2 units. These distances are all so short that crossing over within them can safely be neglected.

Three diploid stocks were planned to furnish these genes in combinations such that the viabilities would be approximately equal. In addition, each stock was made recessive for crossveinless (locus 14.0), to enable us to distinguish the exceptional offspring. The combinations selected were

(a) Yellow crossveinless tan forked;
(b) Ruby crossveinless miniature bar;
(c) Scute bifid crossveinless lozenge dusky.

The total constitution of the required triploid is shown graphically in figure 1, lower three lines.

Two other stocks were needed as "testers." For these the two combinations yellow ruby miniature forked and bifid tan lozenge dusky were selected. Their purpose and use are described below.

SYNTHESIS OF THE REQUIRED TRIPLOID

The first step in the synthesis was to make up the five diploid stocks, a tedious but necessary process. This was facilitated somewhat by the use of L. V. MORGAN's "double-yellow" stock (L. V. MORGAN 1922) for multiplying males at certain stages. Although four months were required in making up these stocks, no unlooked-for difficulties were encountered.

As soon as males of the first stock, *y* c_v *t f*, were available, they were mated to triploid females. Their triploid daughters were again mated to *y* c_v *t f* males. This process was repeated a third time in order to obtain a line of triploids homozygous for *y* c_v *t f*. Unfortunately, when these were obtained, they proved sterile. This difficulty was finally overcome by using one c_v *t f* and two *y* c_v *t f* chromosomes. The non-yellow triploid flies proved fertile and could be used. This stock we have designated as our triploid base. The absence of yellow in one of the chromosomes made it necessary to discard a third of the cultures in later stages of the synthesis when a test could be made for the presence of yellow.

These triploids were mated to ruby crossveinless miniature bar males. Their triploid daughters which showed bar were then mated to scute bifid crossveinless lozenge dusky males. Only about half of their triploid progeny should be of the required constitution. The not-bar triploids obviously lacked an intact chromosome "b" and hence were discarded. The others were crossed with the first tester stock, yellow ruby miniature forked. This test mating, as described below, enabled us to discard the remaining triploids that, because of crossing over, were not of the required constitution.

THE FIRST TESTER STOCK AND ITS USE

Since the data were to be obtained from the exceptional diploid offspring, the X chromosome of the male used at this stage must carry the normal allelomorph of c_v to make possible the recognition of exceptional offspring. As this chromosome was not to enter into any flies which were to be carried further, it might contain any other genes desired. This made it possible to use a stock which would help test the constitution of the triploid female to which it was mated. Since the s_c b_i c_v l_z d_y chromosome had just been introduced into the triploid through the sperm in the last previous mating, it must be present intact in all of the triploids and need

not be tested for. The other two had had an opportunity for crossing over and might not be intact. Likewise, *y* might be absent, due to getting the wrong chromosome from the triploid base stock. The sons would give an indication of the constitution of the triploid, but would be very few in number. By introducing the proper recessive factors through the male, the more numerous intersexes could also be used for deducing the parental constitution. The presence of an intact chromosome "a" could be tested for by means of the end-points *y* and *f*. Likewise, chromosome "b" could be tested for by the two points r_b and *m*, and the presence of bar. The combination *y* r_b *m f* was accordingly used as a tester. Cultures were discarded which failed to show all of the test points *y* r_b *m f* and bar in the males and intersexes.

At this point another difficulty was encountered. Practically none of the triploids gave progeny when mated to males from the tester stock. Low fertility in the tester stock made this stock seem the probable source of the difficulty. So it was discarded, and a new *y* r_b *m f* stock was built up from different sources. It proved much more fertile. The laborious synthesis of the a-b-c triploid then had to be repeated, starting from the triploid base stock, since the later stages could not be retained. Ultimately, several of the crosses were successful, and we were then able to proceed with the tests of the exceptional females that were produced.

THE SECOND TESTER STOCK AND ITS USE

The exceptional diploid females derived from the mating of the triploids with the first tester stock were readily identified by the presence of the character crossveinless. It was then necessary only to identify the constitution of their two X chromosomes. This could be done through their male offspring. Some difficulty was anticipated due to certain characters interfering with the classification of others. Thus, tan cannot be identified in the presence of yellow, nor dusky in the presence of miniature. Bifid was expected to interfere somewhat with miniature and dusky, and bar with lozenge. To overcome these anticipated difficulties a second tester had been made up, of constitution b_t *t* l_z d_y. Males of this stock were used for mating to the exceptional diploid females to be tested. This enabled us to positively identify these four characters in the female offspring. Also, and equally important, the use of the tester gave additional linkage data by which these mutants could be referred to their proper relations in the chromosomes of the mother. Bar, being dominant, could likewise be placed by study of the female offspring. The remaining genes could readily be placed from the male offspring. Counts were not made except in difficult

or unusual cases where there might be some doubt, or in cases where only a small number of offspring were produced.

Due to a shortage of flies of the second tester stock at one time, males from tan vermilion forked or from scute apricot crossveinless tan vermilion forked stocks were substituted in a few of the matings. These proved fairly satisfactory, especially since it was found in practice that bifid does not interfere with miniature and dusky, nor bar with lozenge.

CROSSING-OVER CONTROLS

It was foreseen that in triploid females the distribution of crossing over along the X chromosome might be found to be different from that in diploid females. In order that any observed difference might safely be attributed to the effect of triploidy, rather than to the effect of unknown crossing-over modifiers carried by the particular X chromosomes used, data were collected upon the crossing-over relations shown by these same chromosomes after they had passed through the triploid and emerged again in a diploid condition. Full counts were made of the male offspring of four females, two of which were of constitution a/b, one a/c, and one b/c. The data are given in table 2.

TABLE 2

Control data on linkage relations in chromosomes a, b and c.

TYPE	0	1	2	3	4	1,3	1,4	2,3	2,4	3,4
a/b	196	19	66	43	52	1	5	2	10	2
a/c	68	20	30	14	24	..	..	1	3	1
b/c	75	14	38	16	23	..	..	..	2	..
Totals	339	53	134	73	99	1	5	3	15	3

In addition, partial counts were made of twenty other cultures in the course of establishing the constitution of some of the more unusual exceptions. These counts are summarized in table 3. A summary of the combined data from tables 2 and 3 is given in table 4. The percentages of recombination obtained in these controls are the best basis of expectation

TABLE 3

Partial data on linkage relations in chromosomes a, b and c.

SECTIONS	TOTAL MALES	RECOMBINATIONS	PERCENT
(1) y-r_b	573	31	5.5
(2) r_b-l_a	684	170	24.9
(3) l_a-d_v	720	66	9.2
(4) d_v-B	862	135	15.6

TABLE 4

Summary of control linkage data from tables 2 and 3.

SECTION	TOTAL MALES	RECOMBINATIONS	PERCENT
(1)	1298	90	6.9
(2)	1409	322	22.8
(3)	1445	146	10.1
(4)	1587	257	16.2

in our experiment. They differ slightly from the standard expectations of 7.5, 20.0, 8.7, and 20.8, respectively, for these four sections.

TABULATION OF DATA

Of the triploids tested, six were of the correct constitution for our experiment. One (D) had all genes represented, but through crossing over in the previous generation *B* was combined with *y* c_v *t*, and *f* with r_b c_v *m*. The symbols *f* and *B* were substituted for each other and the data included. Similarly, in H, *y* had become combined with r_b c_v *m B*. Here again a substitution in symbols was made. Thus the eight groups of data can be combined in a single table. A total of 182 exceptional females gave sufficient progeny to permit an identification of their constitution.

The X-constitutions of the 182 exceptional females are given in full in table 5, the data being arranged according to the type of crossing over involved. The letters a, b, and c are used to designate the three non-

TABLE 5

The X-constitution of 2n females each of which has received two X's from the 3n mother.

	3N MOTHER	E	J	A	F	D	I	H	K	TOTALS	
0-0	a-b=*y* c_v *t f*-r_b c_v *m B*	11	4	4	4	3	2	..	..	28	84
	a-c=*y* c_v *t f*-s_c b_i c_v l_z d_y	13	2	5	3	1	2	1	1	28	
	b-c=r_b c_v *m B*-s_c b_i c_v l_z d_y	7	7	2	7	1	3	..	1	28	
0-1	b-*y* s_c b_i c_v l_z d_y	1	1	..	..	..	..	..	..	2	19
	a-s_c r_b c_v *m B*	1	2	..	..	1	..	..	..	4	
	a-b_i c_v l_z d_y	1	1	1	1	..	1	..	..	5	
	b-*y* b_i c_v l_z d_y	..	1	..	..	..	..	..	..	1	
	b-s_c c_v *t f*	1	..	1	1	..	..	..	..	3	
	c-*y* r_b c_v *m B*	..	..	1	..	..	..	..	..	1	
	c-c_v *t f*	1	..	..	..	1	1	..	..	3	
0-2	a-r_b c_v l_z d_y	3	..	..	1	..	..	..	..	4	17
	a-s_c b_i c_v *m B*	2	1	..	..	..	..	..	..	3	
	b-*y* c_v l_z d_y	1	..	..	..	1	..	..	..	2	
	b-s_c b_i c_v *t f*	1	..	1	..	..	..	..	..	2	
	c-*y* c_v *m B*	2	..	..	1	..	1	1	..	5	
	c-r_b c_v *t f*	1	..	..	..	..	..	..	..	1	

TABLE 5 (continued)

	3N MOTHER	E	J	A	F	D	I	H	K	TOTALS	
0-3	a-s_c b_i c_v l_z m B	..	..	..	1	..	..	..	..	1	4
	b-y c_v t d_y	1	..	..	..	..	..	..	..	1	
	b-s_c b_i c_v l_z f	..	..	..	..	1	..	..	..	1	
	c-r_b c_v f	1	..	..	..	..	..	..	..	1	
0-4	a-r_b c_v m	1	1	..	..	..	1	..	..	3	9
	b-y c_v t	1	..	..	..	1	..	..	..	2	
	b-s_c b_i c_v l_z d_y f	..	..	..	..	1	..	..	..	1	
	c-y c_v t B	1	..	..	..	..	..	1	..	2	
	c-r_b c_v m f	1	..	..	..	..	..	..	..	1	
0-1,2	a-s_c r_b c_v l_z d_y	..	1	..	..	..	..	..	..	1	4
	c-y r_b c_v t f	..	..	1	..	..	..	..	..	1	
	c-c_v m B	..	..	1	..	..	1	..	..	2	
0-1, 4	a-s_c r_b c_v m	..	..	2	..	..	..	..	..	2	3
	c-c_v t B	..	1	..	..	..	..	..	..	1	
0-2,4	a-r_b c_v l_z d_y B	1	..	..	..	..	..	..	..	1	3
	b-s_c b_i c_v t	..	..	..	1	..	..	..	..	1	
	c-y c_v m f	1	..	..	..	..	..	..	..	1	
0-3,4	c-r_b c_v B	..	..	..	..	1	..	..	..	1	1
2-2	s_c b_i c_v m B-r_b c_v l_z d_y	..	1	..	..	..	..	..	..	1	1
4-4	y c_v t-s_c b_i c_v l_z d_y f	..	..	1	..	..	..	..	..	1	3
	r_b c_v m-s_c b_i c_v l_z d_y B	..	..	..	1	..	..	..	..	1	
	y c_v t B-r_b c_v m f	..	..	..	1	..	..	..	..	1	
2-2,4	s_c b_i c_v m B-r_b c_v l_z d_y f	..	1	..	..	..	..	..	..	1	1
1-3	s_c r_b c_v m B-y c_v t d_y	..	..	1	..	..	..	..	..	1	2
	y r_b c_v m B-s_c b_i c_v l_z f	..	1	..	..	..	..	..	..	1	
4-1,2	y c_v t B-s_c r_b c_v l_z d_y	1	..	..	..	..	..	..	..	1	1
3-3	r_b c_v d_y-y c_v t m B	..	..	..	..	..	..	1	..	1	1
1-1,4	s_c c_v t f-b_i c_v l_z d_y B	..	..	..	..	..	..	1	..	1	1
	Equationals										
0-0	a-a	..	..	1	..	..	..	..	..	1	1
0-1	a-y b_i c_v l_z d_y	..	1	2	..	..	..	..	..	3	10
	a-y r_b c_v m B	1	..	..	..	..	..	..	..	1	
	b-b_i c_v l_z d_y	..	..	2	..	..	..	..	..	2	
	b-c_v t f	1	..	..	1	1	..	..	..	3	
	c-s_c c_v t f	1	..	..	..	..	..	..	..	1	
0-2	b-s_c b_i c_v m B	1	..	..	..	..	..	..	..	1	3
	b-r_b c_v l_z d_y	..	1	..	..	..	..	..	..	1	
	c-s_c b_i c_v m B	1	..	..	..	..	..	..	..	1	

TABLE 5 (continued)

	3N MOTHER	E	J	A	F	D	I	H	K	TOTALS	
0-3	a-*y* c_v *t m B*	..	..	1	..	..	..	1	..	2	2
0-1,2	a-*y* b_i c_v *m B*	..	..	..	1	..	..	..	..	1	3
	c-s_c r_b c_v *t f*	..	..	..	1	..	..	..	..	1	
	c-b_i c_v *t f*	..	1	..	..	..	..	..	..	1	
0-1,3	c-s_c r_b c_v *f*	..	..	..	..	1	..	..	..	1	1
0-1,4	b-c_v *t*	..	..	1	..	..	..	..	..	1	1
0-2,4	b-s_c b_i c_v *m f*	..	1	..	..	..	..	..	..	1	1
0-1,2,4	c-b_i c_v *m f*	..	..	..	..	1	..	..	..	1	1
1-2	s_c c_v *t f*-*y* c_v l_z d_y	..	1	..	..	..	..	..	..	1	2
	y b_i c_v l_z d_y-*y* c_v *m B*	..	..	..	..	..	1	..	..	1	
1-2,4	b_i c_v l_z d_y-s_c b_i c_v *t B*	..	1	..	..	..	..	..	..	1	2
	s_c c_v *t f*-*y* c_v *m*	..	..	1	..	..	..	..	..	1	
3-1,3	r_b c_v d_y-b_i c_v l_z *m B*	..	1	..	..	..	..	..	..	1	1
	Totals	60	32	29	25	15	13	6	2	182	182

crossover chromosomes, *y* c_v *t f*, r_b c_v *m B*, and s_c b_i c_v l_z d_y, respectively. The individual genes are given for all chromosomes derived from crossing over.

Table 6 presents the same data in a more schematic form, with the letters a, b and c substituted for the factor symbols. The letter indicates the original chromosome from which the particular gene was derived.

THE SOURCES OF THE GENES OF THE EMERGING CHROMOSOMES

The 182 exceptional individuals give a total of 364 distinct X chromosomes, each of which has emerged from an a-b-c triploid mother. A classification of these 364 chromosomes according to the source of the genes shows (summary in table 7) that 69 percent of the emerging chromosomes are identical with an original chromosome; that is, they are either a, b or c, each intact throughout. About 28 percent are composed of sections taken from some two of the three original chromosomes, from a and b, from a and c, or from b and c. These chromosomes have arisen through a process of crossing over. The kinds and frequency of the crossing over will be examined in detail in a following section. Finally, nearly 3 percent of the emerging chromosomes contain parts taken from all three of the

TABLE 6

Generalized constitutions of exceptional offspring of triploid females.

EXCEPTIONS, NON-EQUATIONAL						EQUATIONALS		
0 / 0	a a a a a / b b b b b	28		b b b b b / c c c c a	1	0 / 0	a a a a a / a a a a a	1
	a a a a a / c c c c c	28	0 / 4	c c c c c / a a a a b	2		a a a a a / a c c c c	3
	b b b b b / c c c c c	28		c c c c c / b b b b a	1		a a a a a / a b b b b	1
	b b b b b / a c c c c	2		a a a a a / c b c c c	1	0 / 1	b b b b b / b c c c c	2
	a a a a a / c b b b b	4	0 / 1,2	c c c c c / a b a a a	1		b b b b b / b a a a a	3
0 / 1	a a a a a / b c c c c	5		c c c c c / b a b b b	2		c c c c c / c a a a a	1
	b b b b b / a c c c c	1		a a a a a / c b b b c	2		b b b b b / c c b b b	1
	b b b b b / c a a a a	3	0 / 1,4	c c c c c / b a a a b	1	0 / 2	b b b b b / b b c c c	1
	c c c c c / a b b b b	1	0 / 2,4	a a a a a / b b c c b	1		c c c c c / c c b b b	1
	c c c c c / b a a a a	3		b b b b b / c c a a c	1	0 / 3	a a a a a / a a b b	2
	a a a a a / b b c c c	4		c c c c c / a a b b a	1	0 / 1,2	a a a a a / a c b b b	1
	a a a a a / c c b b b	3	0 / 3,4	c c c c c / b b b a b	1		c c c c c / c b a a a	1
	b b b b b / a a c c c	2	2 / 2	c c b b b / b b c c c	1		c c c c c / b c a a a	1
0 / 2	b b b b b / c c a a a	2	4 / 4	a a a a c / c c c c a	1	0 / 1,3	c c c c c / c b b a a	1
	c c c c c / a a b b b	5		b b b b c / c c c c b	1	0 / 1,4	b b b b b / b a a a c	1
	c c c c c / b b a a a	1		a a a a b / b b b b a	1	0 / 2,4	b b b b b / c c b b a	1

TABLE 6 (continued)

EXCEPTIONS, NON-EQUATIONAL						EQUATIONALS		
	a a a a a / c c c b b	1	2 / 2,4	c c b b b / b b c c a	1	0 / 1,2,4	c c c c c / b c b b a	1
0 – 3	b b b b b / a a a c c	1	1 – 3	c b b b b / a a a c c	1	1 – 2	a c c c c / a a b b b	1
	b b b b b / c c c a a	1		a b b b b / c c c a a	1		c a a a a / a a c c c	1
	c c c c c / b b b a a	1	4 / 1,2	a a a a b / c b c c c	1	1 / 2,4	b c c c c / c c a a b	1
	a a a a a / b b b b c	3	3 – 3	b b b c c / a a a b b	1		c a a a a / a a b b c	1
0 – 4	b b b b b / a a a a c	2	1 / 1,4	c a a a a / b c c c b	1	3 / 1,3	b b b c c / b c c b b	1

TABLE 7

Sources of emerging individual chromosomes.

a	b	c	ab	ac	bc	abc
89	81	82	33	29	40	10
252 (69.3 percent)			102 (28.0 percent)			(2.7 percent)

original chromosomes. These are the most interesting of the emerging chromosomes, since their production involved crossing over, and hence synapsis, of all three of the original chromosomes.

THE KINDS AND FREQUENCIES OF THE CROSSING OVER SHOWN BY THE EMERGING CHROMOSOMES

When the constitutions of the 364 emerging chromosomes are examined and classified with respect to the section or sections in which crossing over has occurred, it is seen (summary in table 8) that about 69 percent of the

TABLE 8

Crossovers shown by emerging chromosomes.

Sections...	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,4
Total	252	36	25	11	16	8	2	5	..	7	1	1
Percent	69.2		24.2	(88)			6.3	(23)				0.3

emerging chromosomes have undergone no apparent crossing over within the distance from yellow to bar. About 24 percent of the chromosomes have resulted from simple crossing over of some two of the original chromosomes. About 6 percent have arisen through double crossing over involving two or three of the original chromosomes. There was one case of triple crossing over that involved all three chromosomes. The nature of these multiple crossings over will be discussed in a following section in more detail.

When the cases of crossing over are summarized to show the total amount that occurred in each crossing-over section (summary in table 9), it is found that the amount of recombination due to crossing over in the

TABLE 9

Summary of crossing over in triploid females.

SECTIONS	TOTAL RECOMBINATION	RECOMBINATION PERCENT	CONTROL PERCENT	RATIO	STANDARD PERCENT
1	52	14.3	6.9	2.07 : 1	7.5
2	41	11.3	22.8	0.50 : 1	20.0
3	14	3.9	10.1	0.39 : 1	8.7
4	30	8.2	16.2	0.51 : 1	20.8

first section is over twice as high as the corresponding percentage for the diploid control data. Throughout the rest of the X the crossing over is only about half the normal amount. It seems probable that the crossing over in the extreme left end is even higher than the high general average for section 1, for two of the 364 chromosomes show crossing over between yellow and scute, and no case of crossing over between yellow and scute has been encountered in diploid material, though several thousand chances have occurred. These two crossovers have settled the serial order of yellow and scute (scute being to the right of yellow), and have given a useful double-recessive stock.

The fact is clear that in the triploid female there is a regional difference of marked extent in the deviations from standard amounts of crossing over for the X chromosome. This indicates that a unit of crossing over at the extreme left end of the X-chromosome map may correspond to a longer section of the chromosome than does a unit of crossing over in regions further to the right. This had already been indicated by the relatively high frequency of mutation per unit of map distance at the extreme left end of the map.

MULTIPLE CROSSING OVER AND COINCIDENCE

The double crossovers (summarized in table 10) are of two distinct types which we shall designate as "recurrent" and "progressive." In the

recurrent type the second crossover takes place between the same two chromosomes as the first, and, as a result, a mid-section from one chromosome becomes intercalated between the ends from another chromosome.

TABLE 10

Double crossing over and coincidence in triploid females.

SECTIONS	RECURRENT	PROGRESSIVE	TOTAL	COINCIDENCE	COINCIDENCE IN CONTROL	STANDARD COINCIDENCE
1,2	6	3	9	1.54	..	.20
1,3	1	1	2	1.00	.15	.45
1,4	4	1	5	1.16	.50	.95
2,3	..	..	..	....	.18	.30
2,4	3	5	8	2.37	.59	.80
3,4	1	..	1	0.87	.30	.40
Total	15	10	25			

In the progressive type the second crossover takes place between chromosomes different from the first and as a result the emerging chromosome is derived from all three original chromosomes. As examples from table 5, the double-crossover chromosome *y* c_v *m f* (a a b b a) is classified as recurrent, while s_c b_i c_v *t B* (c c a a b) is classified as progressive. The 1,2,4 triple crossover (b c b b a) can be analyzed into a 1,2 recurrent and a 2,4 progressive.

If the occurrence of one crossover does not prejudice the manner of occurrence of the second crossover these two types should be equally frequent. As the summary of table 10 shows, there were 15 recurrent and 10 progressive crossovers. This is not a statistically significant departure from equality. That half, or nearly half, of the double crossovers were of the progressive type signifies that, in general, two strands that have crossed over with each other were as free to cross over with a strand from the third chromosome as with a strand from one of the two original chromosomes. That is, all three chromosomes must be substantially on a par with one another in the manner of synapsis. This is good evidence that synapsis in the triploid female, generally, if not always, involves all three chromosomes equally throughout their length, and does not follow the type that seems to obtain for the sex chromosomes of an XXY female, namely, complete synapsis between two with the third left unsynapsed.

The coincidence values, calculated from the combined recurrent and progressive double crossovers, all involve high probable errors and are therefore not greatly to be relied upon (table 10). The surprising feature is their high values as compared with corresponding indices for control and for standard data. In three of the classes the occurrence of one cross-

over seems actually to have favored the occurrence of a second (coincidence over 1.0). A decrease in crossing over accompanied by a rise in coincidence shows that it is not simply the frequency of crossing over that is reduced (which would give lowered crossing over with unchanged proportion of doubles among those that do occur), but that the average distance apart of those that do occur is reduced.

THE GENES ASSOCIATED IN EMERGING CHROMOSOMES

Having examined the emerging chromosomes individually as to the source of their genes and as to the types of crossing over, we may next examine the characteristics of the chromosomes that have emerged together. From the types of "association" we should be able to deduce the types of distribution that have been produced by the two maturation divisions. Of the 182 exceptional flies that were examined as to the nature of the associated chromosomes, 28 (rightmost column of table 6) were found to have chromosomes that were identical for some part of their lengths. They possessed two representatives of certain genes of which the mother possessed only one. They are called "equational" exceptions, since they are of the type that would be produced by failure of the separation of sister strands of the tetrad (or hexad in this case).

The most significant point in connection with these equational exceptions is that they are not necessarily identical *throughout* their length. One may be an original chromosome throughout and the other be a crossover composed of a part of that same chromosome joined to a supplementary part from a second chromosome. Furthermore, in case one is a crossover the other is not likewise an identical crossover at the same point. *This means that at the time crossing over occurred each of the original chromosomes had already become split into two strands.* If crossing over occurred before each original chromosome had thus split, then the two strands must necessarily be identical throughout their length, and if one were derived by crossing over at a particular point between a given two original chromosomes, then the other must be an identical crossover at that point between the same two chromosomes. As the analysis shows, at the time of crossing over there are present in the triploid female six strands, and sister strands can undergo crossing over, each with a different other strand. This conclusion had already been indicated in the study of equational exceptions from diploid females (BRIDGES 1916) and is confirmed by the study of crossing over in attached-X females (ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT, unpublished) and by the study of equational exceptions from X-rayed females (unpublished work of ANDERSON).

On random distribution of the six strands present before maturation, a given strand will be associated with its sister strand in one-fifteenth of the cases, and all equational cases should total three-fifteenths or 20 percent of the cases. This calculation disregards the fact that a given strand loses its original constitution through crossing over, so that it would be more correct to say that for any given point, e.g., the locus of yellow, the equationals are expected to be 20 percent of the total. When the associated chromosomes are tabulated with respect to each of the five points followed (table 11), it is seen that the values for all the different points are far below this expectation. That is, there is a strong tendency for genes in sister strands to pass to opposite poles rather than to remain together at the same pole. But this tendency is a graded one. At point V

TABLE 11

Associations of genes in emerging chromosomes.

	I	II	III	IV	V	EXPECTATIONS
aa	9	5	3	1	1	12.1
bb	8	1	2	2	1	12.1
cc	4	4	. . .	. .	. .	12.1
Subtotal	21	10	5	3	2	36.4
Percent	11.5	5.5	2.7	1.6	1.1	20.0
ab	51	60	59	60	56	48.5
ac	55	59	59	61	62	48.5
bc	55	53	59	58	62	48.5
Subtotal	161	172	177	179	180	145.6
Percent	88.5	94.5	97.3	98.4	98.9	80.0

(*f*,*B*,+) there were only two cases of association of sister genes, which gives 1.1 percent of association instead of the twenty percent expected. For the points further to the left the percentage is progressively higher, being 11.5 at point I (*y*,s_c,+). This seriation shows that the two ends of the X chromosome are markedly different with respect to separation of sister genes: at the extreme left the association is fairly frequent, but probably at the extreme right it does not occur at all. For all except two cases, crossing over has occurred to the right of the homozygous section, and in these two cases (the *y* c_v *t f* and the c_v *m B* equationals) crossing over between forked and the right end (some 13.5 units still further to the right than forked) may well have occurred. This indicates that the right end of the chromosome is the end governing the separation of the strands, for

which point the first[2] division is invariably reductional. The second division then invariably separates sister strands and the percentage of association for the extreme right end is zero. But whenever crossing over occurs, the part of one strand distal to the point of crossing over (that is, to the left) is replaced by a strand from another homolog. Then at the first division, the separation for the extreme right end, and for all points between it and the point of crossing over is reductional and for the remainder is equational. At the end of the first division in the triploid female the egg nucleus that will give an exception includes four strands, one of which, a a a b b, is a crossover between two original strands, and is accompanied by a full b b b b b strand. The other two strands are the strands reciprocal to the first, namely, a a a a a and b b b a a, or are both c c c c c. In case the other two strands are both c c c c c, then the second division will give either $\frac{a\ a\ a\ b\ b}{c\ c\ c\ c\ c}$ or $\frac{b\ b\ b\ b\ b}{c\ c\ c\ c\ c}$, either of which gives an ordinary-type exception. But in case the other two strands are the reciprocals then the second division will result in $\frac{a\ a\ a\ a\ a}{b\ b\ b\ b\ b}$, or $\frac{a\ a\ a\ a\ a}{a\ a\ a\ b\ b}$, or $\frac{b\ b\ b\ a\ a}{b\ b\ b\ b\ b}$, or $\frac{b\ b\ b\ a\ a}{a\ a\ a\ b\ b}$. Two of these equally probable possibilities result in equationals, one results in complementary crossover strands and one results in different non-crossovers. The further to the left a given point lies, the more numerous should be the cases in which crossing over between the given point and the extreme right end would occur and give opportunity for an equational exceptional to arise.

The reason that the separation begins at the right end is presumably that the separating mechanism, the spindle fiber, is attached to that end. This conclusion has also been reached by a study of the seriation in the frequency of equationals that occur in attached-X cases (ANDERSON 1925, L. V. MORGAN 1925, and unpublished work of STURTEVANT). We may therefore regard it as established that the spindle fiber is attached at the right end and that the left end is the free, outer, or distal end of the X chromosome.

THE CROSSING-OVER TYPES PRESENT IN ASSOCIATED CHROMOSOMES

As we have seen, a given gene that was present only once in the mother can come out in both X's of a daughter only if crossing over occurs between

[2] As far as the genetic data show, it could equally well be assumed that it is the second division that is reductional. To avoid lengthy discussion, we have here followed through only the case in which the reduction separation occurs at the first division, as is clearly the case in the male.

its locus and the point of spindle-fiber attachment, and accordingly the constitution of associated chromosomes with respect to the genes is not independent of their constitution with respect to crossing over. The analysis just given, together with table 11, was primarily concerned with the origin of the genes of the associated chromosomes. In the paragraph below, the associated chromosomes will be examined primarily with regard to the types of crossing over shown. There are four sections within each of which it can be determined whether a crossover has occurred or not, and in case it has, which chromosomes have contributed the parts of the emerging crossover chromosome. Table 5 is arranged according to the crossover formulae of the emerging chromosomes.

There were 84 cases in which the two associated chromosomes were both non-crossovers throughout their length.

There were 19, 17, 4 and 9 cases in which one chromosome was a complete non-crossover and the other chromosome was derived from the other two by crossing over in sections 1, 2, 3 and 4, respectively.

There were a few cases in which the second chromosome had come from double crossing over.

An especially interesting type of case is that in which the two associated chromosomes were both crossovers at the same point between the same two chromosomes, and were, in fact, complementary products of the same crossing over (2-2; 4-4; 2-2,4).

There were a few cases in which both chromosomes were crossovers, but in different sections (1-3; 4-1,2). One strand of a given chromosome (a) crossed over with one strand from a second chromosome (b) at one place, while the other a-strand crossed over with a strand from the third chromosome (c).

Finally, there is the special case, differing from the above only in that one a-strand crossed over with a b-strand in a certain section, while the other a-strand crossed over with a c-strand in that same section, though most probably not at the same point within the section (3-3; 1-1,4).

Within the equationals approximately the same types of chromosomes with respect to crossing over were encountered. In the one case where both chromosomes were identical non-crossovers throughout the sections followed, it is inferred that one of them had undergone crossing over to the right of point V, that is, between bar and the spindle-fiber attachment. The $\frac{b\ b\ b\ b\ b}{c\ c\ b\ b\ b}$ individual likewise involved crossing over between point V and the right end.

These relations are generalized in table 6, where the space between consecutive symbols represents the crossing-over section, and the symbols give the source of the genes that emerge on the two sides of the crossing-over section. In table 12 is a summary of the cases of the different types with respect to each crossing-over section.

TABLE 12

Associations of crossovers in emerging chromosomes.

TYPE OF ASSOCIATIONS		FORMULA	1	2	3	4	TOTAL	PERCENT
Two non-crossovers, different....		$\frac{a\ a}{b\ b}$	126	140	167	153	586	80.5
Crossover and dissimilar non-crossover.....		$\frac{a\ b}{c\ c}$	29	28	8	23	88	12.1
Crossover and similar non-crossover.....	(a)	$\frac{a\ b}{a\ a}$	16	7	2	1	26	3.6
	(b)	$\frac{b\ a}{a\ a}$	5	2	..	..	7	1.0
Two crossovers, complementary..		$\frac{a\ b}{b\ a}$	..	2	1	3	6	0.8
Two crossovers, different.....		$\frac{a\ b}{c\ a}$	1	..	1	..	2	0.3
Two non-crossovers, identical....		$\frac{a\ a}{a\ a}$	5	3	3	2	13	1.8
Two crossovers, identical.....		$\frac{a\ b}{a\ b}$	..	..	..	..	..	0.0

Thus, if we let a represent a gene from any given chromosome (either a, b or c) and b represent a gene from either one of the other two chromosomes, then by far the most frequent type of association was that in which both strands were non-crossovers, but the second strand was from a different original chromosome than the first (formula $\frac{a\ a}{b\ b}$.)

The next most frequent case was that in which one strand was a crossover between two different original chromosomes and the other strand came from the third original chromosome $\left(\frac{a\ b}{c\ c}\right)$.

Another type of case is that in which a crossover strand is associated with a non-crossover strand from one of the two chromosomes involved

in the crossing over. This type of association gives rise to equational exceptions. The above general type has two subtypes, namely, that in which the homozygous gene is to the left of the crossover point (formula $\frac{a\ b}{a\ a}$) and that in which it is to the right of the crossover point (formula $\frac{b\ a}{a\ a}$). On chance, these two types should be equal, but it was found that they were clearly unequal, in that 26 were $\frac{a\ b}{a\ a}$ while only 7 were $\frac{b\ a}{a\ a}$. A consideration of the spindle-fiber attachment has shown that equationals only arise when a crossing over occurs somewhere to the right of a given gene; therefore, the equationals should all be of the $\frac{a\ b}{a\ a}$ type, and none of the $\frac{b\ a}{a\ a}$ type. The assumed discrepancy is only apparent, for closer examination of these seven cases shows that in each of them the original situation had been disturbed by a crossing over of one or the other strand still further to the right.

The association of a crossover chromosome with a non-crossover similar to one of its own parts $\left(\frac{a\ b}{a\ a}\right)$ should be half as frequent on chance as its association with the third chromosome $\left(\frac{a\ b}{c\ c}\right)$. This holds approximately only for the leftmost section, and is about one-fourth for sections 2 and 3, while only one of the 24 crossovers of section 4 was of the $\frac{a\ b}{a\ a}$ type. An important comparison may be had when only the rightmost crossovers are considered. These are summarized in table 13. The ratios of the $\frac{a\ b}{c\ c}$ to the $\frac{a\ b}{a\ a}$ type of association are practically the same as when all crossovers are considered (table 14). The very low frequency of the $\frac{a\ b}{a\ a}$ type for region 4 may well indicate the absence of this type of association at the extreme right end, that is, near the spindle-fiber attachment. The gradually increasing frequency toward the left may be accounted for, at least in part, by the increasing possibility of crossing over further to the right in the chromosome which supplies the left-hand section of the observed crossover chromosome. In such cases the $\frac{a\ b}{a\ a}$ type of association may be

derived by the same process which gives rise to the $\frac{a\ b}{c\ c}$ type. Any such increase in the $\frac{a\ b}{a\ a}$ type of association should be accompanied by a slight decrease in the $\frac{a\ b}{c\ c}$ type. In the absence of interference, the percent of invalidity of the left crossover section, due to crossing over to the right

TABLE 13

Association of crossovers in emerging chromosomes; rightmost crossovers only.

TYPE OF ASSOCIATION	FORMULA	1	2	3	4	TOTAL
Two non-crossovers, different	$\frac{a\ a}{b\ b}$	84	113	142	153	492
Crossover and dissimilar non-crossover	$\frac{a\ b}{c\ c}$	19	24	6	23	72
Crossover and similar non-crossover	$\frac{a\ b}{a\ a}$	10	4	2	1	17
Two crossovers, complementary	$\frac{a\ b}{b\ a}$	..	1	1	3	5
Two crossovers, different	$\frac{a\ b}{c\ a}$	..	..	1	..	1

TABLE 14

Comparison of seriation of ratios of $\frac{a\ b}{c\ c}$ *to* $\frac{a\ b}{a\ a}$.

TYPE OF ASSOCIATION	1	2	3	4	TOTAL
All crossovers (table 12)	1.8	4	4	23	3.4
Right-hand crossovers (table 13)	1.9	6	3	23	4.2

of the observed crossover, should equal the percent of crossing over between the region and the spindle-fiber attachment. Allowing for six percent of crossing over to the right of forked and bar, the invalidity would amount to about 10 percent for the mid-point of region 4, 16 percent for region 3, 24 percent for region 2, and 36 percent for region 1. Interference would reduce the percentage, especially in the right-hand regions, whereas the high frequency of double crossing over where longer regions are involved, as shown by the extremely high coincidence values, should give a marked increase in the left-hand regions. Crossing over to the right of point V would similarly invalidate either chromosome.

Another type of association is that of complementary crossovers, $\frac{a\ b}{b\ a}$. This type is expected to be half as numerous as the $\frac{a\ b}{a\ a}$ type, and a quarter as numerous as the $\frac{a\ b}{c\ c}$ type. In fact, it was far below either of these expectations, having been only one-fifteenth as numerous as the $\frac{a\ b}{c\ c}$ type. When only the right-hand crossovers are considered (table 13), the proportions remain the same. There is a suggestion of a seriation increasing from left to right.

An unknown, but probably small, proportion of the cases that are apparently $\frac{a\ b}{b\ a}$ may have been really examples of another type, namely, one in which each of the two strands of a given original chromosome has crossed over somewhere within a given crossing-over section. Thus, if a strand from a given chromosome crosses over with the sister strand from the second within that same section, then it is possible for an a b strand to be associated with a b a strand without the two having been complementary products of a single crossing over. If each of two sister strands crosses over within a given section with a strand from a different other chromosome, then an $\frac{a\ c}{b\ a}$ type of association can arise. Two clear cases of this were found, and this fact is a strong argument that all three X chromosomes were in synapsis throughout their length, and not simply that the left ends of two were synapsed and the right end of the third was synapsed with the right end of one of the first two. This argument is the stronger the shorter the section in which both strands crossed over with strands from different other chromosomes. Section 3 seems short enough to meet this requirement, and one of the two cases occurred there.

Cases in which both associated strands are identical non-crossovers ($\frac{a\ a}{a\ a}$) arise when there has been crossing over in some section to the right of that considered. They are equationals. Only a few such cases were found, and this fact shows that most of the seriation present in the proportions of homozygosis (table 11) was not due to accumulated crossovers, but rather due to the seriation from right to left in the ratio of $\frac{a\ b}{a\ a}$ to $\frac{a\ b}{c\ c}$ association.

A very significant feature is the total absence of the association of identical crossovers $\frac{a\ b}{a\ b}$. This class would be expected to occur freely if crossing over occurred before the splitting of the chromosomes, whereas the $\frac{a\ b}{a\ a}$ and $\frac{a\ b}{c\ a}$ classes would be impossible. The frequent occurrence of the latter classes together with the total absence of the $\frac{a\ b}{a\ b}$ class proves that crossing over does not occur until after each original chromosome has split into two strands, each of which then follows its own separate course during the crossing-over process.

The question arises as to the possibility of crossing over between the two strands that are derived from the same original chromosome. Since such crossovers do not lead to recombination of linked characters, they cannot be detected directly. If they occur with a frequency equal to that of crossing over between any two other strands, one-third of the total crossing over in the diploid would be undetectable, whereas in the triploid it would be only one-fifth. A systematic increase of approximately 20 percent in the observed crossing over in the triploid might then be expected. However, the specific changes in crossing over in the triploid, that is, the reduction in regions 2, 3 and 4, and the large increase in region 1 mask any difference that might be due to crossing over between sister strands.

SUMMARY

In order to study crossing over in the triploid condition, triploid females of *Drosophila melanogaster* were synthesized in such a way that five points in each of their three X chromosomes could be identified in their progeny. The X constitution of these triploids is represented graphically in figure 1 (page 420). Only the exceptional diploid daughters are included in the data. These contain two X chromosomes derived from their triploid mother and none from their father. The advantage of using the exceptional daughters is that their two X chromosomes were derived from a single oöcyte. The constitutions of 182 exceptional daughters were identified and are given in detail in tables 5 and 6.

Of the chromosomes present in the exceptional daughters, 69 percent were non-crossovers, 28 percent were crossovers involving two of the three original chromosomes, and about 3 percent were crossovers involving all three of the parental X chromosomes.

Crossing over in the region from yellow to bifid was twice as high as in the diploid controls. In the regions to the right of bifid it was only one-half as high.

Two types of double crossing over were found: a recurrent type in which the second crossover takes place between the same two chromosomes as the first, and a progressive type in which the second crossover takes place between chromosomes different from the first. The latter type gives a chromosome derived from parts of all three original chromosomes. Of the double crossovers obtained, 15 were recurrent and 10 progressive. This shows that synapsis generally involves all three X chromosomes and that a crossover in one region does not markedly prejudice which strands may cross over in another region.

Coincidence values are unusually high, indicating that double crossing over occurs more freely than in the diploid condition.

Of the 182 exceptional daughters examined, 28 had X chromosomes identical in some part of their lengths. Such females are known as equational exceptions. In only one case did the two X chromosomes seem identical throughout. The remaining cases were identical for a part of their length only. This shows that at the time crossing over occurred each of the original chromosomes had already become split into two strands.

The percentage of equationals was lowest for the right end, being 1.1 for point V ($f\ B+$). It increased progressively toward the left, reaching 11.5 percent for point I ($y\ s_c\ +$). Random assortment should give 20 percent of equationals for any point. It is believed that the separation of strands is controlled by spindle fibers attached to the extreme right end, at which point the first division would be always reductional, and that equationals occur as a result of crossing over. The progressive increase toward the left, in the percentage of equationals, is interpreted as due to an accumulation of crossovers of the type which gives rise to equationals.

The crossovers present in associated chromosomes are classified and summarized in table 12 and discussed in the accompanying text. Several of the relative frequencies depart widely from the frequencies expected on random assortment. Further evidence is adduced to show that crossing over does not occur until each of the chromosomes has become split into two strands.

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GENETIC FACTORS FOR YELLOW PIGMENT IN MAIZE AND THEIR LINKAGE RELATIONS[1]

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INTRODUCTION

The geneticist working with the chlorophyll pigments of plants is rapidly discovering that their genetic complexity parallels their chemical intricacy. In maize, for example, more than a score of fundamentally different genes are already known to comprise the hereditary complex of these pigments. Most of these directly control the green components of the leaf pigment, as evidenced by their genetic behavior with respect to albino and virescent seedlings. In addition, there are not less than fifteen hereditary factors which influence the distribution or the pattern of chlorophyll in the plant, but these are not considered as affecting the pigment itself, fundamentally. Heretofore, only one gene has been reported that directly affects the yellow components, the xanthophyll or carotinoid pigments. A second gene involving these yellow colors has now been found, and its genetic behavior and linkage relations determined.

THE L_1 FACTOR

Genetic behavior toward albino genes

The first factor controlling yellow pigments in maize was reported in 1917 by the writer (LINDSTROM 1917) and its genetic reaction with other

[1] Paper No. 7 from the Department of Genetics, IOWA STATE COLLEGE, Ames, Iowa.

genes reported in greater detail in two later papers (LINDSTROM 1918, 1921). In these reports it was symbolized by the letter *l* (from the Latin *luteus*). The symbol has now been changed to l_1 so as to differentiate it from the second factor involved in the genetics of the yellow pigments.

Gene l_1 governs the formation of a distinct yellow color which may be apparent in the mature plant or in the seedling stage. Its actual appearance in the mature plant is demonstrated in the *Japonica* type of striping, common in ornamental maize plants. When gene l_1 is present, such *Japonica* plants have alternate stripes of green and yellow, whereas when L_1 is present the stripes are green and white. Gene l_1 is best known in the seedling stage, where with the proper genotype it develops into a distinct pure-yellow seedling.

Its interactions with other known seedling genes have been reported earlier (LINDSTROM 1921), but they may be summarized as follows, being different from those of the second gene for yellow:

L_1W_1V,	green
l_1W_1V,	green
L_1W_1v,	virescent-white
L_1w_1V,	white or albino
L_1w_1v,	white or albino
l_1W_1v,	virescent yellow
l_1w_1V,	pure yellow
l_1w_1v,	pure yellow

In addition the relations between l_1 and other albino genes, w_2 and w_3 (LINDSTROM 1924), are reported herewith. It may also be added that l_1 is borne on the same chromosome as the *R* aleurone factor and the *g* chlorophyll factor for the golden type of plant. Some of its newer linkage relations, notably with w_2 and d_f (defective endosperm) are reported here for the first time.

When l_1 and the three different recessive albino genes w_1, w_2 and w_3[2] are introduced into the same plant by means of appropriate hybridization methods, so that a tetra-heterozygous plant of the composition $L_1l_1W_1w_1W_2w_2W_3w_3$ is produced, the following interaction of factors takes place:

$L_1W_1W_2W_3$,	green seedlings
$l_1W_1W_2W_3$,	green seedlings

[2] This albino gene, w_3, is the same as that reported in a previous paper (LINDSTROM 1924). It has not as yet been tested against the albino gene of the same number, described by DEMEREC (1923).

$L_1w_1W_2W_3$,	white seedlings
$L_1W_1w_2W_3$,	white seedlings
$L_1W_1W_2w_3$,	white seedlings
$L_1W_1w_2w_3$,	white seedlings
$L_1w_1W_2w_3$,	white seedlings
$L_1w_1w_2W_3$,	white seedlings
$L_1w_1w_2w_3$,	white seedlings
$l_1w_1W_2W_3$,	yellow seedlings
$l_1W_1w_2W_3$,	yellow seedlings
$l_1W_1W_2w_3$,	yellow seedlings
$l_1W_1w_2w_3$,	yellow seedlings
$l_1w_1W_2w_3$,	yellow seedlings
$l_1w_1w_2W_3$,	yellow seedlings
$l_1w_1w_2w_3$,	yellow seedlings

The above factorial interaction is merely a complex modification of the 12:3:1 ratio, the fundamental relation being as follows:

12	9	$L\,W$,	green
	3	$l\,W$,	green
3	3	$L\,w$,	white
1	1	$l\,w$,	yellow

Before the data that shall prove the above situation are discussed, it might be well to call attention to two general relations expressed by this factorial interaction. First, there are two pure genetic types of green plants, one with L_1 and the other with l_1. The former never gives yellow seedlings in F_2 when crossed with albino-carrying greens, whereas the latter always does. This has been abundantly proven and verified many times. Second, none of the albino genes, which apparently are only related to the green components of the plant pigment, are capable of inhibiting the development of yellow color as they do the green pigment. In other words, their effect is entirely restricted to the green pigment, since the yellow pigment is always produced by l_1l_1 whenever w_1, w_2 or w_3 are present in a homozygous condition. This obviously indicates a certain independence of the two types of pigments. This may have a general physiological meaning, especially when we shall consider later that the second yellow gene acts otherwise with respect to these same albino genes.

Data to prove the factorial interaction noted above come largely from a cross involving the following parental genotypes:

$yy\ L_1l_1\ W_1w_1\ W_2W_2\ W_3W_3\ D_fD_f \times YY\ L_1L_1\ W_1W_1\ W_2w_2\ W_3w_3\ D_fd_f$

In other words, the pistillate parent was a white-seeded, green plant which, when self-fertilized, gave a seedling progeny of 12 green : 3 white : 1 yellow,

TABLE 1

Individual F_2 progenies showing seedling segregation. P_1 genotypes, $L_1l_1W_1w_1W_2W_2W_3W_3 \times L_1L_1W_1W_1W_2w_2W_3w_3$.

F_1 GENOTYPES	PEDIGREE NUMBER	F_2 SEGREGATION					
		Observed			Expected (based on 21.7 percent crossing over between L_1 and W_3)		
		Green	White	Yellow	Green	White	Yellow
$L_1W_1W_2W_3 \cdot L_1W_1W_2W_3$	5950	108	..	..	..	..	..
and	5956	88	..	..	..	..	..
$l_1W_1W_2W_3 \cdot L_1W_1W_2W_3$	5984	144	..	..	..	..	..
	5985	158	..	..	..	..	..
Total		498	..	..	498	..	..
$L_1w_1W_2W_3 \cdot L_1W_1W_2W_3$	5347	99	33	..	..	..	..
	5970	225	54	..	..	..	..
	5981	118	40	..	..	..	..
Total		442	127	..	427	142	..
$L_1W_1W_2W_3 \cdot L_1W_1w_2W_3$	5974	102	37	..	..	..	..
	5978	204	59	..	..	..	..
Total		306	96	..	301	101	..
$L_1W_1W_2W_3 \cdot L_1W_1W_2w_3$	5965	117	40	..	..	..	..
	5967	176	72	..	..	..	..
	5969	202	56	..	..	..	..
	5972	142	51	..	..	..	..
Total		637	219	..	642	214	..
$L_1w_1W_2W_3 \cdot L_1W_1w_2W_3$	5952	184	110	..	..	..	..
	5955	154	123	..	..	..	..
	5968	125	118	..	..	..	..
	5973	157	112	..	..	..	..
	5976	98	74	..	..	..	..
Total		718	537	..	706	549	..
$L_1w_1W_2W_3 \cdot L_1W_1W_2w_3$	5337	50	46	..	..	..	..
	5339	40	31	..	..	..	..
	5345	58	31	..	..	..	..
	5349	76	71	..	..	..	..
	5962	149	113	..	..	..	..
Total		373	292	..	374	291	..

TABLE 1 (continued)

F_1 GENOTYPES	PEDIGREE NUMBER	F_2 SEGREGATION					
		Observed			Expected (based on 21.7 percent crossing over between L_1 and W_3)		
		Green	White	Yellow	Green	White	Yellow
$L_1W_1W_2W_3 \cdot L_1W_1w_2w_3$	5342	116	81	..	..	..	..
	5951	168	123	..	..	..	..
	5983	163	123	..	..	..	..
Total		447	327	..	435	339	..
$L_1w_1W_2W_3 \cdot L_1W_1w_2w_3$	5971	121	156	..	117	160	..
$l_1w_1W_2W_3 \cdot L_1W_1W_2W_3$	5343	72	14	3	..	..	..
	5348	126	36	10	..	..	..
	5954	204	47	11			
	5960	264	60	20			
	5979	241	70	22	..	..	..
Total		907	227	66	900	225	75
$l_1W_1W_2W_3 \cdot L_1W_1W_2w_3$	5963	178	36	14	171	43	14
$l_1W_1W_2W_3 \cdot L_1W_1w_2W_3$	5338	267	72	3	..	..	..
	5341	192	48	2	..	..	..
	5961	356	90	5	..	..	..
Total		815	210	10	804	220	11
$l_1w_1W_2W_3 \cdot L_1W_1W_2w_3$	5344	164	97	20	..	..	..
	5959	149	77	24	..	..	..
	5964	168	83	26	..	..	..
	5966	203	98	47	..	..	..
Total		684	355	117	651	379	127
$l_1w_1W_2W_3 \cdot L_1W_1w_2W_3$	5335	121	71	12	..	..	..
	5346	145	74	31	..	..	..
	5958	165	105	21	..	..	..
	5977	154	98	20	..	..	..
Total		585	348	84	572	372	73
$l_1W_1W_2W_3 \cdot L_1W_1w_2w_3$	5336	183	125	23	..	..	..
	5340	175	94	24	..	..	..
	5953	202	99	23	..	..	..
Total		560	318	70	533	347	68
$l_1w_1W_2W_3 \cdot L_1W_1w_2w_3$	5957	125	130	28	..	..	..
	5975	122	134	37	..	..	..
	5980	133	138	50	..	..	..
Total		380	402	115	378	415	104

whereas the staminate parent was a yellow flint type which, when self-fertilized, would give 9 green : 7 albino seedlings. The F_1 generation consisted of 80 green plants, 50 of which were self-fertilized. Fifty F_2 seedling progenies were grown in the greenhouse with the results shown in table 1.

A summary of these F_2 data has been arranged in table 2 in such a manner as to show that the F_1 genotypes, of which there would be expected 16 in number, were all realized among the 50 progenies resulting

TABLE 2

Summary of F_2 seedling progenies from a cross with the parental genotypes $L_1l_1W_1w_1W_2W_2W_3W_3 \times L_1L_1W_1W_1W_2w_2W_3w_3$.

F_1 GENOTYPES	F_2 PROGENIES		
	Seedling ratio	Observed	Calculated
$L_1W_1W_2W_3 \cdot L_1W_1W_2W_3$	All green	4	6.250
$l_1W_1W_2W_3 \cdot L_1W_1W_2W_3$	All green		
$L_1w_1W_2W_3 \cdot L_1W_1W_2W_3$	3 green : 1 white	3	3.125
$l_1w_1W_2W_3 \cdot L_1W_1W_2W_3$	12 green : 3 white : 1 yellow	5	3.125
$L_1W_1W_2W_3 \cdot L_1W_1W_2w_3$	3 green : 1 white	4	3.125
$l_1W_1W_2W_3 \cdot L_1W_1W_2w_3$	12 green : 3 white : 1 yellow	1	3.125
$L_1w_1W_2W_3 \cdot L_1W_1W_2w_3$	9 green : 7 white	5	3.125
$l_1w_1W_2W_3 \cdot L_1W_1W_2w_3$	36 green : 21 white : 7 yellow	4	3.125
$L_1W_1W_2W_3 \cdot L_1W_1w_2W_3$	3 green : 1 white	2	3.125
$l_1W_1W_2W_3 \cdot L_1W_1w_2W_3$	12 : 3 : 1, modified by linkage	3	3.125
$L_1w_1W_2W_3 \cdot L_1W_1w_2W_3$	9 green : 7 white	5	3.125
$l_1w_1W_2W_3 \cdot L_1W_1w_2W_3$	36 : 21 : 7, modified by linkage	4	3.125
$L_1W_1W_2W_3 \cdot L_1W_1w_2w_3$	9 green : 7 white	3	3.125
$l_1W_1W_2W_3 \cdot L_1W_1w_2w_3$	36 : 21 : 7, modified by linkage	3	3.125
$L_1w_1W_2W_3 \cdot L_1W_1w_2w_3$	27 green : 37 white	1	3.125
$l_1w_1W_2W_3 \cdot L_1W_1w_2w_3$	108 green : 111 white : 37 yellow, modified by linkage	3	3.125
	$\chi^2=8.366$ $P=0.87$		

therefrom. The approach to the expected proportion of these numerous F_1 genotypes (as shown by their F_2 behavior) proved to be surprisingly close, the probability that the deviations were merely due to errors of random sampling, being 87 percent. This in itself is fairly significant, but taken in conjunction with the proof offered by the individual F_2 progenies, the evidence becomes indisputable. Thus we may be reasonably confident that gene l_1 has the characteristic interactions with genes w_1, w_2 and w_3 in physiological development, as is demonstrated by the factorial interaction on a 12 : 3 : 1 basis.

Linkage of L_1 and W_2

A detailed analysis of table 1 will show that gene l_1 is most certainly linked in inheritance with one of the albino genes. This situation was predicted some time ago when l_1 was proved to be linked with the aleurone factor R and the chlorophyll factor G, and also when R and W_2 were shown to belong to the same linkage group (LINDSTROM 1924).

The method of isolating the particular albino gene concerned in this linkage has been rendered easy because of the complete linkage between w_2 and an endosperm defect controlled by the factor d_f. The latter was present in the staminate parent of the original cross. Another verification of this was provided by the fact that gene w_1 was linked with endosperm color (Yy) also involved in the cross.

Linkage data involving l_1 and w_2 may be found in table 1 in four different sets of progenies, with the following genotypes:

Dihybrid,	$l_1W_2 \,.\, L_1w_2$,	Pedigree Nos. 5338, 5341, 5961.
Trihybrid,	$l_1w_1W_2 \,.\, L_1W_1w_2$,	Pedigree Nos. 5335, 5346, 5958, 5977.
Trihybrid,	$l_1W_2W_3 \,.\, L_1w_2w_3$,	Pedigree Nos. 5336, 5340, 5953.
Tetrahybrid,	$l_1w_1W_2W_3 \,.\, L_1W_1w_2w_3$,	Pedigree Nos. 5957, 5975, 5980.

In other words, there will be progenies of a di-, tri- and tetrahybrid nature, all involving l_1 and w_2 in various combinations with the other chlorophyll genes. In every case the two linked genes must exhibit the "repulsion" phase of linkage.

The dihybrid situation, $l_1W_2 \,.\, L_1w_2$, will produce a seedling progeny whose 12 : 3 : 1 ratio of seedling types will be modified by the linkage. The three F_2 progenies (5338, 5341 and 5961), thus modified, gave a total of 815 green : 210 albino : 10 yellow seedlings. Classification in all cases was easy because the types were so clearly differentiated. The above is a wide deviation from a 12 : 3 : 1 ratio, but with linkage between l_1 and w_2 giving 21.7 percent crossing over (gametic ratio of 1 : 3.6) the theoretical expectancy becomes 804 : 220 : 11, a distribution that is surprisingly similar to the actual data. The closeness of fit in this case is very good, χ^2 being much less than one (0.697). Standing alone, this fit proves nothing, of course, but the following series of progenies give ample verification for it.

There are two trihybrid situations involving l_1 and w_2, one including w_1, and the other w_3. Four progenies were isolated among the 50 F_2 progenies in this category that showed a linkage between w_1 and y, proving that w_1 and not w_3 was concerned. The genotype of these four progenies (5335, 5346, 5958, 5977) may be expressed as $l_1w_1W_2W_3 \,.\, L_1W_1w_2W_3$.

Were there no linkage, these should give a seedling ratio of 36 green : 21 white : 7 yellow. Actually, there were 585 : 348 : 84, respectively. If 21.7 percent crossing over is assumed for the l_1-w_2 linkage, the expected proportion becomes 572 : 372 : 73, which gives a value for P of 0.18 on the closeness-of-fit basis.

The other trihybrid situation involving W_3w_3 was discovered in the F_2 progenies 5336, 5340 and 5953. Here 560 green : 318 white : 70 yellow seedlings were realized, where 533 : 347 : 68 would be expected with the same intensity of linkage assumed above. The value for P becomes 0.15 when the goodness of fit is determined.

The tetrahybrid situation which involved all the four pairs of genes, may serve as the real verification for the linkage of l_1 and w_2 on the basis of 21.7 percent crossing over. Pedigree Nos. 5957, 5975 and 5980 gave in the F_2 generation a total of 380 green : 402 albino : 115 yellow. Without linkage this ratio would be on the basis of 108 : 111 : 37 among 256 individuals. Obviously, the actual data do not fit such a ratio. But with linked inheritance the expectancy becomes 378 : 415 : 104, which is a good approach to the experimental data. The value for P is 0.47 in this case, and it affords a verification for the situation in the simpler dihybrid situation.

In all of the cases quoted above the agreement between observed and expected is disturbed noticeably by a consistent deficiency among the white or albino seedlings. In all these progenies w_2 is involved, and it is this albino gene which is completely linked with a recessive endosperm defect, d_f, that most certainly gives a lower percentage of germination than that obtained from the normal seed. The constant deficiencies in all the cases reported above are logically explained on this basis. Because of the variable nature of this recessive endosperm defect, it has not been deemed advisable to attempt any correction for differential germination caused thereby. The only harm caused by this situation is the relative inexactness of the crossover percentage between L_1 and W_2. However, it is not thought that a percentage of crossing over of 21.7 is more than two percent off.

There is, accordingly, ample proof and verification for the fact that l_1 and w_2 belong in the same linkage group. On the modern chromosome theory of heredity these genes are about 22 units apart on the chromosome. A discussion of the gene arrangement on this chromosome is reserved for a later section of this paper, since the second gene for yellow pigment also belongs here.

It is worthy of note that l_1 was also found to be linked with d_f, the endosperm-defective factor that is completely linked with w_2. Only occasionally was a yellow seedling observed in the progeny from defective seeds. This rare occurrence is explicable on the basis that l_1 and D_f came into the hybrid from the same parent, and only the occasional crossover would give the yellow seedling from defective endosperm. Because of the difficulty in classifying this endosperm character accurately, a calculation of its crossover percentage with other genes has been abandoned.

THE L_2 FACTOR

Genetic behavior towards albino genes

The second gene that controls yellow pigment in maize was isolated by Mr. M. T. JENKINS of the U. S. DEPARTMENT OF AGRICULTURE, in the course of his inbreeding experiments with commercial varieties of maize. It appeared in the first inbred generation of C. I. No. 133, a yellow dent strain. It proved to be a simple recessive to green. Mr. JENKINS generously provided seed of this type for tests against the original yellow-producing factor.

The yellow seedlings are distinctly deeper in color than those produced by gene l_1. The actual pigment has not yet been analyzed. Its chemical nature and its physiological behavior should prove to be intensely interesting when compared with the pigment formed by l_1, because the two genes have an entirely different genetic behavior towards the genes controlling the green components of chlorophyll. This yellow-seedling type exhibits the sharpest and most non-variable segregation among the many chlorophyll variations dealt with in ten years of experience.

Where l_1 gives a 12 : 3 : 1 interaction with W_1w_1, for example, l_2 exhibits a 9 : 3 : 4 relation as follows:

9 L_2W_1,	green
3 l_2W_1,	yellow
3 L_2w_1,	white
1 l_2w_1,	white

The striking feature of this factorial interaction is that a physiological relationship apparently exists here between the green and yellow pigment-producing factors. Whereas l_1 acts independently of w_1 in development, l_2 does not. The effect of l_2 is entirely inhibited by w_1, indicating, perhaps, a closer organic relationship between the two than exists between l_1 and w_1. This would seem to argue for the presence of two sorts of carotinoid pigments in maize, one that is related to the green pigments in physio-

logical development, the other not,—at least the genetic evidence would suggest that such a prediction might be realized when the physiologist or chemist attacks the problem.

The 9 : 3 : 4 interaction involving l_2 and one albino gene is demonstrated by the data in table 3. Here, seven progenies, arising from mother ears of the composition $L_2w_1 \cdot l_2W_1$ or $L_2w_3 \cdot l_2W_3$, gave a total of 910 green : 304 yellow : 425 white seedlings. With independent inheritance on a 9 : 3 : 4 basis the expected distribution becomes 922 : 307 : 410, respectively, which approaches the actual results closely ($\chi^2 = 0.73$). The individual progenies themselves are in excellent agreement with such a hypothesis.

TABLE 3

Seedling progenies showing the 9 : 3 : 4 ratio arising from mother plants of the composition $L_2w_1 \cdot l_2W_1$ *or* $L_2w_3 \cdot l_2W_3$.

PEDIGREE NUMBER	GREEN	YELLOW	WHITE
6620	115	42	62
6630	146	60	75
6633	184	60	76
6635	122	37	50
6661	95	23	45
6663	146	46	68
6672	102	36	49
Total	910	304	425
Expected	*922*	*307*	*410*

$\chi^2 = 0.73$

This fundamental 9 : 3 : 4 relationship also occurs in the more complex progenies that result from such genotypes as $L_2l_2W_1w_1W_2w_2$, $L_2l_2W_1w_1W_3w_3$, and $L_2l_2W_1w_1W_2w_2W_3w_3$. A discussion of these situations is reserved for a future report involving the interactions of the two yellow-producing genes themselves, as well as the linkage between L_2 and W_2 which is so close that much larger progenies are required to test it critically.

Linkage of L_2 and R

Preliminary observations have shown beyond reasonable doubt that the new yellow-producing gene belongs in the same linkage group as the first one, namely, in the L_1-*R*-*G* group. The evidence proving this comes from crosses of the original inbred strain that was heterozygous for the yellow-seedling type by some pedigreed inbred stocks carrying aleurone

color, stem color and certain albino genes. Some of the F_2 progenies from these crosses naturally were heterozygous L_2l_2, and had at the same time 9 : 7 ratios of aleurone color. Incidentally, the anthocyanic stem-color characters produced by the multiple-allelomorphic situation R^g, r^g and r^r (EMERSON 1921) were involved also.

Seven self-pollinated ears of the genetic composition $L_2R^gCA \,.\, l_2r^rcA$ are available for a determination of the relations between the second yellow-producing gene and the R factor. Their progenies have been arranged in tables 4 and 5. All of them gave indisputable evidence of linkage involving both aleurone and stem color. It is rather interesting to observe that the stem-color situation calls for the "repulsion" phase, and the aleurone relation gives the "coupling" phase of linkage in the very same progenies.

TABLE 4

Seedling progenies from mother ears with the genotype $L_2R^g \cdot l_2r^r$, showing stem-color and chlorophyll segregation with linkage between L_2 and R.

PEDIGREE NUMBER	WITH RED STEM COLOR		WITH NON-RED STEM COLOR		CROSSOVER PERCENTAGES
	Green L_2r^r	Yellow l_2r^r	Green L_2R^g	Yellow l_2R^g	
6613	138	54	58	10	37.2
6614	135	58	58	10	33.3
6618	154	77	65	2	21.7
6621	147	60	66	16	35.8
6636	132	43	58	11	41.5
6638	176	84	82	11	24.4
6645	104	29	46	9	45.0
Total	986	405	433	69	33.9±0.7

The stem-color linkage involves a simple dihybrid arrangement, the red stems of the seedlings being caused by r^r and the non-reds by R^g; while the L_2l_2 factor pair gives a similar, single-factor difference. It is to be remembered that, whereas R^g is dominant with respect to aleurone color, it is recessive to r^r in the matter of stem color. Thus, four classes arise: green seedlings with red stems, green seedlings with non-red stems, yellow with red stems, and the double-recessive class, yellow seedlings with non-red stems. Seven such distributions are listed in table 4.

In each progeny there is a noticeable linkage between these two factor pairs, with the usual variation in the individual crossover percentages. The totals show 33.9±0.7 percent crossing over between L_2l_2 and the

stem-color factors. It is interesting to compare this percentage with that obtained from the aleurone distribution which follows (table 5).

The aleurone situation arising from the genotype $L_2RCA \,.\, l_2rcA$ gives a trihybrid interaction on a 27 : 9 : 21 : 7 basis, which in this case is modified by linkage. Table 5 illustrates this linked inheritance between one of the aleurone factors and the yellow-producing gene. The aleurone factor involved in the linkage is *R* and not *C*, as is proven by the stem-color linkage described previously.

TABLE 5

Seedling progenies from mother ears with the genotype $L_2RCA \cdot l_2rcA$, possessing 9 : 7 aleurone ratios and segregating for yellow seedlings, showing linkage between L_2 and R.

PEDIGREE NUMBER	FROM COLORED ALEURONE		FROM COLORLESS ALEURONE		CROSSOVER PERCENTAGES
	Green L_2R	Yellow l_2R	Green L_2r*	Yellow l_2r*	
6613	122	33	74	31	39.2
6614	115	22	78	46	27.8
6618	126	28	93	51	31.4
6621	123	28	90	48	32.3
6636	113	22	77	32	36.7
6638	150	46	108	49	40.0
6645	88	18	62	20	43.3
Total	837	197	582	277	35.4 ± 0.7

* Also includes *Rc*.

The seven progenies in table 5 are also variable in their individual crossover percentages. The average percent in this case proved to be 35.4 ± 0.7. This intensity of linkage should be exactly comparable with that given by the stem-color distribution, namely 33.9 ± 0.7. Why there should be any difference between these two percentages may not be evident. If R^g, r^r and r^g are truly multiple allelomorphs, there should be no difference, theoretically. However, a plausible explanation lies in the slight uncertainty attendant on stem-color determinations in the seedling stage. They are not fool-proof, although the segregation in this particular material seemed to be unusually clean-cut. It was observed, however, that stem color in the yellow-seedling class was sometimes but faintly developed on the weaker seedlings. The stem-color ratio involved in table 4 proved to be 1391 red : 502 non-red, when 1420 : 473 were to be expected. Unquestionably some faintly-developed reds were overlooked and classified as non-reds, occasionally, a situation that is especially natural among the

green seedlings. Accordingly, the data in table 4 may not be as reliable as those in table 5, since the aleurone segregation in the latter seemed to be unusually distinct. Perhaps a crossover percentage of 35.0 might represent the truer value of the linkage between L_2 and R.

ARRANGEMENT OF THE LINKED GENES

It is not desirable to give a complete chromosome map of this second linkage group at the present time. In a future report a great deal of data involving this group will be presented, and the details fully established. Previous experiments show that R and L_1 are almost completely linked, one experiment indicating about 1.6 percent crossing over (LINDSTROM 1921). R and W_2 have also been shown to be linked (LINDSTROM 1924), and in this paper L_1 and W_2, as well as R and L_2, are proven to be linked.

The order of these genes can be given as L_1-R-W_2-D_f-L_2. In the next report, which will appear shortly, the relations between L_1 and L_2, as well as those between D_f and both L_1 and L_2 are to be established. This second linkage group in maize has the additional factors g (golden), l_{i1} (lineate) and p_{g1} (pale green, BRUNSON 1924), thus making a total of eight linked genes on this chromosome.

DISCUSSION

Perhaps the most interesting contribution in this article is the proof of the existence of two distinct genes for yellow pigments in maize, each having a different factorial interaction with the albino genes. The gene l_1 gives a 12 : 3 : 1 ratio with either of the three albino genes, whereas the new l_2 gene shows a 9 : 3 : 4 relationship. This certainly points to two different physiological relations between the green and yellow components of plant pigments. The 12 : 3 : 1 ratio indicates that the yellow- and green-producing genes have no common relation in development, the double-recessive yellow class l_1w, demonstrating that the albino gene does not inhibit the yellow pigment. In the 9 : 3 : 4 situation, however, the double-recessive albino class l_2w at once indicates that these two genes have a common relation, because the gene w does in this case inhibit the yellow pigment normally produced by l_2.

Such a genetic relationship may argue for a certain physiological behavior between the green and yellow pigments of plants. In fact it would be interesting to verify the prediction that two yellow pigments may be found in plant tissue, one of which is organically related to the green pigment and the other not. The stage is now set for the plant physiologist or the chemist to push the inquiry further. Material is at hand for a

critical test of this, since we have been able to separate by genetic means the two types of yellow pigment in a pure form, that is, free from any trace of the green chlorophyll pigment. Whether or not pure carotin and xanthophyll have been isolated, remains to be determined by the chemist.

The other contribution of this paper is merely a routine determination of some linkages between these yellow-producing genes and other factors in the same linkage group. It is rather interesting to note that the two yellow genes happen to reside on the same chromosome, some 35 units apart. Three other chlorophyll genes (g, l_{i1} and p_{g1}) also belong here.

SUMMARY

Two genes responsible for the formation of yellow pigments in maize have now been discovered. Both are recessive in inheritance, and strictly follow Mendelian inheritance.

Gene l_1 interacts with each of the three albino genes w_1, w_2 and w_3 so as to give a 12 : 3 : 1 ratio, whereas l_2, the new yellow gene, reacts with these same albino genes to produce a 9 : 3 : 4 relationship. This is thought to indicate a fundamental, physiological (or chemical) difference in the yellow pigments of maize, so that one yellow pigment is considered as being independent of the green components of chlorophyll in development and the other not.

Linkage tests show that both genes are borne on the same chromosome. Genes L_1 and W_2 proved to be about 22 units apart, and L_2 and R approximately 35 units apart. This provides for an arrangement of these factors in this second linkage group in maize as follows: L_1-R-W_2-L_2.

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TWO CASES OF CLOSE LINKAGE IN THE JAPANESE MORNING-GLORY

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INTRODUCTION

In the course of genetical experiments with the Japanese morning-glory (*Pharbitis Nil*), I have reported over a dozen cases of linkage (IMAI 1919, 1921, 1924). Some of these cases exhibited such a high degree of linkage that, the observed specimens being comparatively few in number, they seemed at first to show the monohybrid ratio 3 : 1, or 1 : 2 : 1. In the present paper I shall discuss two such cases of close linkage.

CONTRACTA AND WHITE-MARGINED FLOWER

A contracted state of growth of the plant is one of the distinct characters found in some varieties of the Japanese morning-glory; the leaves are contracted, and have a thick lamina and a polished surface; the stem is thick and brittle; the plant has somewhat contracted flowers; and produces smaller seeds than those of the normal plant. These characteristics are due to the multiple expressions of the single "*contracta*" factor. Speaking in general, the traits of contractedness appear all over the plant growth. The breeding experiments show that *contracta* behaves as a simple character recessive to the normal growth.

The white-margined flower is also commonly found in our cultivated varieties. The genetic behavior of the white-margined corolla, however, is not simple. So far as the present paper is concerned, the whiteness of the flower margin acts as a dominant allelomorph to the self-colored condition. But it is sometimes produced by more than one factor, and behaves also either as a dominant or a recessive factor in inheritance.

When we consider these allelomorphic pairs together in the hybrid segregation, they show a high degree of linked assortment. The frequency of crossing over is about 1 percent (IMAI 1919, 1921).

The experimental results which I now have in hand, are those obtained from the coupling cross. When the double dominant, normal with white-margined flower, is crossed with the double recessive, *contracta* with colored flower, the resultant F_1 assumes the characteristics of the former parent. In the next generation the segregation occurred in four types, making an unusual arrangement in their frequency. The actual numbers obtained are shown in table 1. The result shows obviously a case of segre-

TABLE 1

F_2 segregation from crosses between normal white-margined and contracta self-colored morning-glories.

CROSS	NORMAL WITH WHITE-MARGINED FLOWER	NORMAL WITH SELF-COLORED FLOWER	*Contracta* WITH WHITE-MARGINED FLOWER	*Contracta* WITH SELF-COLORED FLOWER	TOTAL
323×316	36	0	0	9	45
323a×316	65	1	1	14	81
324×316	245	1	0	70	316
D9 selfed	79	1	0	22	102
Total	425	3	1	115	544
Expected (on the basis of 133.05:1)	*406*	*2*	*2*	*134*	*544*

$\chi^2 = 4.58$ $P = 0.21$

gation with close linkage. The single-dominant classes were made up of small numbers of individuals, while the double-recessive plants were produced in large numbers. On calculation of the linkage value the result shows 0.75 percent of crossing over. Rearing the F_3 generation, I obtained the results summarized in tables 2[1] and 3. The families which segregated in the dihybrid scheme, gave, without exception, the coupling data, just like those segregated in the preceding generation. To obtain the average value, the available coupling data have been collected in table 4.

Estimating the linkage value from the above numbers we obtain the gametic ratio of 90.91 : 1, the frequency of crossing over being 1.09 per-

[1] The data are not strictly of the F_2 offspring, but of the second segregating generation of the natural hybrids. So far as the present paper is concerned, the data, however, can be treated in a similar way with those of F_2 obtained from the controlled cross.

TABLE 2

The F_2 data of the cross, 324×316.

CHARACTER OF F_1	PEDIGREE NUMBER	NORMAL WITH WHITE-MARGINED FLOWER	NORMAL WITH SELF-COLORED FLOWER	*Contracta* WITH WHITE-MARGINED FLOWER	*Contracta* WITH SELF-COLORED FLOWER	TOTAL
	Total of 19 families	669				669
	2	17	1	0	3	21
	3	54	1	0	11	66
	4	15	0	0	4	19
	5	10	0	0	6	16
	6	48	0	0	13	61
	9	27	1	0	14	42
	10	13	0	0	7	20
	11	7	0	0	4	11
	12	7	0	0	3	10
	14	21	0	0	3	24
	15	12	0	0	5	17
	16	25	0	0	4	29
Normal	17	8	0	0	3	11
with	18	15	0	0	6	21
white-	20	10	0	0	3	13
margined	21	42	0	0	11	53
flower	23	28	0	0	14	42
	26	3	0	0	2	5
	29	28	0	0	10	38
	31	3	0	0	3	6
	32	29	0	0	9	38
	35	12	0	0	7	19
	36	52	0	0	20	72
	37	22	2	0	7	31
	38	29	2	0	7	38
	39	5	0	0	1	6
	40	6	0	0	1	7
	42	36	0	0	9	45
	48	5	0	0	1	6
	49	14	0	0	5	19
	50	3	0	0	1	4
	51	11	0	0	6	17
	52	4	0	0	3	8
	Total	622	7	0	206	835
	Expected (on the basis of 117.43:1)	*622.75*	*3.50*	*3.50*	*205.25*	*835*
Contracta with self-colored flower	Total of 17 families				428	428

$\chi^2=7.00$ $P=0.07$

TABLE 3

The data of the second segregating generation of a natural hybrid, D9.

CHARACTER OF F_2	PEDIGREE NUMBER	NORMAL WITH WHITE-MARGINED FLOWER	NORMAL WITH SELF-COLORED FLOWER	*Contracta* WITH WHITE-MARGINED FLOWER	*Contracta* WITH SELF-COLORED FLOWER	TOTAL
	Total of 19 families	647				647
	42	23	4			27
	Expected	*20.25*	*6.75*			*27*
	14	6		1		7
	Expected	*5.25*		*1.75*		*7*
Normal with white-margined flower	1	11	0	0	3	14
	3	7	1	0	5	13
	4	17	0	0	6	23
	5	16	0	1	10	27
	11	25	0	0	12	37
	12	62	0	0	21	87
	15	22	0	0	10	32
	16	50	1	0	11	62
	22	10	0	0	6	16
	24	38	0	0	9	47
	26	9	0	0	2	11
	29	18	0	0	6	24
	33	47	0	2	24	73
	34	17	1	0	3	21
	40	24	1	0	4	29
	43	13	0	0	2	15
	44	13	0	0	6	19
	45	28	0	0	7	35
	46	10	1	0	6	17
	47	50	1	0	10	61
	48	16	0	0	4	20
	49	30	0	0	12	42
	51	9	0	0	4	13
	52	17	0	0	3	20
	53	7	0	0	4	11
	55	44	0	1	18	63
	57	27	0	0	9	36
	60	18	0	1	1	20
	91	10	0	0	1	11
	62	41	0	1	11	53
	Total	706	6	6	230	948
	Expected (on the basis of 77.54 : 1)	*705*	*6*	*6*	*231*	*948*
Contracta with self-colored flower	Total of 13 families				393	393

$\chi^2 = 0.006$ $P = 1.0-$

TABLE 4

Summary of all data showing the coupling phase of segregation involving normal versus contracta habit and white-margined versus self-colored flowers.

SOURCE	NORMAL WITH WHITE-MARGINED FLOWER	NORMAL WITH SELF-COLORED FLOWER	*Contracta* WITH WHITE-MARGINED FLOWER	*Contracta* WITH SELF-COLORED FLOWER	TOTAL	PERCENTAGE OF CROSSOVER
From table 1	425	3	1	115	544	0.75
From table 2	622	7	0	206	835	0.84
From table 3	706	6	6	230	948	1.27
Total	1753	16	7	551	2327	1.09
Expected (on the basis of 90.91:1)	*1733.75*	*11.50*	*11.50*	*570.25*	*2327*	

$\chi^2=4.39$ $P=0.23$

cent. Owing to the high linkage value an apparent discrepancy might be expected in the genotypic ratio of the double dominants,—the normals with white-margined flowers,—when a genotypic comparison is attempted. If the estimated value is applied to the gametic ratio, the actual expectation will be as indicated in table 5.

TABLE 5

A comparison of the observed ratio with the expected ratio on the basis of linkage (100 : 1) and independent segregation.

GENOTYPE	THEORETICAL RATIO CALCULATED ON THE BASIS OF 100 : 1	NORMAL RATIO	OBSERVED FREQUENCY			EXPECTED FREQUENCY	
			324×316	D9	TOTAL		
DDFF	10,000 (50)	(1)	19	19	38	33.88	
DDFf	200 (1)	(2)	0	1	1	0.68	$\chi^2=1.15$
DdFF	200 (1)	(2)	0	1	1	0.68	
DdFf (*DF*×*df*)	20,000 (100)	(2)	33	30	63	67.76	$P=0.88$
DdFf (*Df*×*dF*)	2 (0+)	(2)	0	0	0	0.01	
Total	30,402 (152)	(9)	52	51	103	103.01	

If the factor for *contracta* is represented by *d*, and its dominant allelomorph by *D*, the white-margined and self-colored flowers by *F*[2] and *f*, respectively, the double heterozygotic composition, *Dd Ff*, may be produced by a mating of *DF*×*df* or by *Df*×*dF*. Although these two sorts of double heterozygote present no different features in the phenotype, they

[2] In Japanese, the white-margined condition of the corolla is called generally by the name of "Fukurin" or "Hukurin."

should give rise to divergent modes of segregation in the subsequent generation. The segregation of zygotes from the mating $DF \times df$ should show the coupling phase, while those derived from the cross $Df \times dF$ should give the repulsion phase. The theoretical frequency of occurrence of these two types of heterozygotes derived from a single original cross is about 10,000 : 1, as has already been shown; in other words, we should find on the average only one individual among ten thousand heterozygotes examined which would represent a reversal of phase from coupling to repulsion or *vice versa*. The number of heterozygotes tested, however, was actually far less than the ten thousand required to give an even chance of finding one such reversal of phase. From this consideration, it is quite reasonable to conclude that in the examination of such a small number of families, none would be found representing the repulsion phase of segregation.

YELLOW LEAF AND BROWNISH FLOWER

Strictly speaking, the so-called yellow leaf is yellowish-green in color, and it is preferred by cultivators on account of the soft appearance of its leaves. The distinction between the two types with green and yellow leaves is sharp even in the cotyledons. Though the yellow strain makes a luxuriant growth under suitable conditions, it may be overrun by more vigorous green plants; and so we often have a discrepancy in the segregating ratio, when both forms are planted side by side. Breeding experiment shows that the green leaf is transmitted as a unit dominant to the yellow one.

The Japanese morning-glory exhibits abundant variations in flower color. Among them, the brownish flower is allelomorphic with the non-brownish flower, the former being recessive to the latter.

MIYAZAWA (1918, 1921) crossed the green-leafed brownish-flowered[3] with the yellow-leafed white-flowered form carrying the non-brownish color factor. Selfing the green-leafed and non-brownish-flowered F_1 plants thus obtained, he raised the next generation consisting of three phenotypes: the double dominant, the one single dominant and the other single dominant, in the proportion of 2 : 1 : 1, with no double-recessive type so far as his data showed. To explain such an unusual result he proposed the hypothesis of "factor interrelation."[4] According to my opinion, his result

[3] Denoted as "dark-red" in MIYAZAWA'S (1918) paper.

[4] In his first paper MIYAZAWA (1918, p. 82) states: "if we denote the one parent by *GGDD* and the other by *ggdd*, there exists the interrelation between the factors *G* and *D*, inasmuch as in the presence of *D* the production of the dark-red flower-colour takes place when *G* is present in homozygous condition, and that of the red (magenta or scarlet) colour, when *G* is present in heterozygous condition or altogether absent. . . . ."

is nothing but the expression of close linkage in the repulsion phase. Examining carefully MIYAZAWA'S data, I suggested in an earlier paper (IMAI 1921) the application of the linkage hypothesis, and later proved it with my own data (IMAI 1924). For the investigation of the factorial behavior in such a case of close linkage it is advisable to examine the coupling data rather than those of repulsion. From this point of view, I made experiments of the coupling cross on a considerable scale, and in this way determined the linkage value.

Coupling cross

From a packet of seeds bought from a seedsman in the previous year I obtained in 1920 three families segregated into two pairs of allelomorphs, the yellow-leaf set and the brownish-flower one. Owing to the unknown origin of the material, I cannot tell from what crosses these families were actually derived. Judging from the mode of segregation, however, it may be presumed that they were all doubly heterozygous. Further, from their coupling segregations, the families may be considered to have originated from the union of two gametes *GR* and *gr*, where *g* denotes the factor for the yellow leaf, and *r* the factor for the brownish flower. The actual segregation is presented in table 6. The infrequency of the middle classes shows close linkage between the two pairs of factors under discussion.

TABLE 6

Segregations in the progenies of three self-fertilized plants grown from commercial seeds.

PEDIGREE	GREEN-LEAFED NON-BROWNISH-FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH-FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
D5 selfed	53	0	1	28	82
D111 selfed	15	0	0	9	24
D112 selfed	23	0	1	10	34
Total	91	0	2	47	140

In 1922, I had a number of F_1 plants from a controlled cross, green-leafed red-flowered × yellow-leafed brownish-flowered. The three pure strains, named HT, 50 and AG, respectively, were used in hybridizing the other constant strain designated as M1. The three former have the double-dominant characters, while the latter is characterized by the double-recessive traits. The F_1 plants bore green leaves and red flowers, and on self-fertilization produced the F_2 population shown in table 7.

TABLE 7

Segregations in F_2 from crosses between green-leafed red-flowered and yellow-leafedbrownish-flowered.

CROSS	GREEN-LEAFED NON-BROWNISH FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
M1×HT−1	22	1	0	2	25
−2	86	1	1	39	127
−3	52	0	0	10	62
−4	27	0	0	7	34
−5	58	0	0	18	76
−6	25	0	0	4	29
−7	42	1	0	6	49
M1×50−1	28	0	1	10	39
−2	53	0	0	20	73
M1×AG−1	96	0	0	23	119
−2	53	1	1	29	84
Total	542	4	3	168	717
Expected (on the basis of 101.20 : 1)	*534.25*	*3.50*	*3.50*	*175.75*	*717*

$\chi^2 = 0.72$ $P = 1.0-$

These three matings showed close agreement in mode of segregation, and accorded well with the results obtained in the hybrid families derived from commercial seeds, as described above. There is, thus, definite evidence for the assumption that close linkage occurs between the genes for these two character pairs. The linkage value calculated from the frequency of crossing over shown by these F_2 data is 0.98 percent.

From two matings, M1×50 and M1×AG, I raised F_3-generation progenies in order to determine the genotypic constitution of the individual F_2 plants. The results obtained are summarized in tables 8 and 9. As there are no fundamental differences in the contents of these two tables we may include them both in the following discussion. The F_3 families from the double-dominant green-leafed red-flowered F_2 individuals are grouped, excepting two families, into two categories, namely, those which show no segregation and those which segregate with respect to both character pairs. Of the two exceptional cases, family No. 20 (of the cross M1×50) segregated merely in the leaf color, while No. 70[5] (of the cross M1×AG)

[5] The number being too small I traced the F_4 generation of these five plants to know their actual composition, and experimentally observed no yellow leaves in their offspring. As a result of this examination we can state that the segregation of this family is not the result of any sort of deviation of the dihybrid assortment.

TABLE 8

The F_3 data of the cross, M1×50.

CHARACTER OF F_2	PEDIGREE NUMBER	GREEN-LEAFED NON-BROWNISH-FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH-FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
	Total of 19 families	397				397
	20	32		8		40
	Expected	*30*		*10*		*40*
	1	19	0	0	5	24
	3	13	0	0	5	18
	4	53	0	0	9	62
	5	18	0	0	5	23
	14	36	2	0	5	43
	15	6	0	0	2	8
	16	29	0	0	9	38
	23	17	1	0	8	26
	27	60	1	0	17	78
	28	6	0	0	4	10
	31	28	0	1	8	37
	32	48	0	0	10	58
	34	13	0	0	3	16
	36	2	0	0	1	3
	39	42	0	0	18	60
Green-leafed non-brownish-flowered	40	47	0	1	14	62
	44	29	0	0	7	36
	45	24	0	0	5	29
	47	20	0	0	4	24
	49	31	0	0	14	45
	56	11	0	0	2	13
	58	28	0	1	11	40
	60	31	0	0	5	36
	62	5	0	0	4	9
	63	34	0	0	11	45
	65	4	0	0	4	8
	66	21	0	0	4	25
	67	7	0	0	3	10
	70	64	0	0	14	78
	71	41	0	0	8	49
	72	7	0	0	2	9
	73	19	0	2	6	27
	75	56	0	0	12	68
	76	74	1	1	29	105
	77	84	0	0	34	118
	78	29	0	0	7	36

TABLE 8 (continued)

CHARACTER OF F_2	PEDIGREE NUMBER	GREEN-LEAFED NON-BROWNISH-FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH-FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
	80	21	0	1	3	25
	81	32	1	0	8	41
	84	34	0	0	15	49
	86	13	0	0	6	19
	87	37	0	0	17	54
	88	55	0	0	11	66
	89	35	1	0	7	43
	Total	1283	7	7	376	1673
	Expected (on the basis of 117.90 : 1)	*1247.75*	*7*	*7*	*411.25*	*1673*
Green-leafed brownish-flowered	74		23		9	32
	Expected		*24*		*8*	*32*
Yellow-leafed brownish-flowered	Total of 25 families				260	260

$\chi^2 = 4.00$ $P = 0.26$

exhibited a monohybrid segregation in the flower color only. Such rare occurrence of segregation in monohybrid ratios is clearly the result of close linkage. If we attempt to estimate the linkage value from those F_3 families which showed dihybrid segregation, the frequency of crossing over is 0.84 percent from table 8, and 1.19 percent from table 9. To determine the value as precisely as can now be done, all of the available coupling data are collected in table 10. Here the calculation shows that the frequency of crossing over is 1.01 percent. In other words, we have a confirmation of the earlier conclusion, that there is only one chance of obtaining a crossover gamete in about every hundred germ cells of the double-heterozygotic hybrids. With such a discrepancy in the gametic frequency there should be found theoretically in the double-dominant F_2 a genotypic ratio like that shown in table 11.

TABLE 9

The F_3 data of the cross, M1×AG.

CHARACTER OF F_3	PEDIGREE NUMBER	GREEN-LEAFED NON-BROWNISH-FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH-FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
	Total of 20 families	353				353
	70	4	1			5
	Expected	*3.75*	*1.25*			*5*
	2	11	1	0	4	16
	6	12	0	1	2	15
	16	10	0	0	5	15
	18	52	0	0	19	71
	19	7	1	0	2	10
	20	9	0	1	3	13
	23	9	1	0	1	11
	24	1	0	0	2	3
	25	12	0	0	1	13
	28	39	0	1	12	52
	30	9	0	0	3	12
	35	5	0	0	2	7
	40	36	0	0	2	38
	42	16	0	0	10	26
	44	25	1	0	9	35
	47	24	0	0	4	28
Green-leafed non-brownish-flowered	48	101	1	0	29	131
	49	39	0	1	12	52
	51	63	0	0	16	79
	52	44	1	0	12	57
	53	2	0	0	2	4
	54	26	0	0	7	33
	55	28	1	0	4	33
	56	16	0	0	5	21
	61	46	0	0	9	55
	63	15	0	0	9	24
	66	85	0	1	30	116
	68	48	0	0	11	59
	71	7	0	0	4	11
	72	14	0	1	4	19
	73	21	0	1	7	29
	76	125	0	1	34	160
	Total	957	7	8	276	1248
	Expected (on the basis of 83.10 : 1)	*928.50*	*7.50*	*7.50*	*304.50*	*1248*
Yellow-leafed brownish-flowered	Total of 23 families				214	214

$\chi^2=3.61$ P=0.31

TABLE 10

Summary of all dihybrid coupling data involving green leaves versus yellow leaves and brownish flowers versus non-brownish flowers.

CROSS	GREEN-LEAFED NON-BROWNISH-FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH-FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
D5 selfed	53	0	1	28	82
D111 selfed	15	0	0	9	24
D112 selfed	23	0	1	10	34
(M1×HT) F_2	312	3	1	86	402
(M1×50) F_2	81	0	1	30	112
F_3	1283	7	7	376	1673
(M1×AG) F_2	149	1	1	52	203
F_3	957	7	8	276	1248
Total	2873	18	20	867	3778
Expected (on the basis of 98.13 : 1)	*2814.50*	*19*	*19*	*925.50*	*3778*

$\chi^2 = 5.02$ $P = 0.17$

TABLE 11

A comparison of the observed ratio with the expected ratio on the basis of linkage (100 : 1) and of independent segregation.

GENOTYPE	THEORETICAL RATIO CALCULATED ON THE BASIS OF 100 : 1	NORMAL RATIO (NO LINKAGE)	OBSERVED FREQUENCY	EXPECTED FREQUENCY (100 : 1)	
GGRR	10,000 (50)	(1)	29	32.24	
GGRr	200 (1)	(2)	1	0.64	$\chi^2 = 0.83$
GgRR	200 (1)	(2)	1	0.64	$P = 1.0-$
GgRr (*GR*×*gr*)	20,000 (100)	(2)	67	64.47	
GgRr (*Gr*×*gR*)	2 (0+)	(2)	0	0.01	
Total	30,402 (152)	(9)	98	98.00	

This table shows that such an expectation corresponds adequately with the actual results.[6] Of F_2 plants which were considered to be derived from the contribution of a crossover gamete, there was actually only one case, namely, No. 74, of the cross, M1×50, which bore green leaves and brownish flowers. This F_2 plant gave rise to a monohybrid segregation as

[6] In making this table I omitted generally from the observed totals the families which consisted of inadequate numbers of offspring.

to leaf color, and, without doubt, may be considered to have been derived from the combination of a crossover gamete *Gr* and a non-crossover gamete *gr*.

Repulsion cross

In 1920 I made a hybridization of the yellow-leafed red-flowered form (SG) and the green-leafed brownish-flowered form (324). The F_1 plants from this cross bore green leaves and red flowers. The two F_2 progenies are given in table 12.

TABLE 12

F_2 segregation showing the repulsion phase in the cross SG×324.

CROSS	GREEN-LEAFED NON-BROWNISH-FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH-FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
SG×324−1	21	8	6	0	35
−2	34	16	19	0	69
Total	55	24	25	0	104

The segregation took place in three phenotypes nearly in the proportion of 2 : 1 : 1, the result being in perfect accord with MIYAZAWA'S (1918) experiment. Such a segregation is to be expected as a consequence of close linkage. Here we expect, theoretically, one double-recessive plant in about forty-thousand offspring; so that experimentation on a considerable scale is needed, if we are to obtain this double-recessive individual in the dihybrid segregation of the repulsion cross. The 2 : 1 : 1 ratio of segregation was also observed when I examined the F_3 progenies of five double-dominant F_2 plants; the results are presented in table 13. With

TABLE 13

The F_3 data of the cross SG×324.

PEDIGREE NUMBER	GREEN-LEAFED NON-BROWNISH-FLOWERED	GREEN-LEAFED BROWNISH-FLOWERED	YELLOW-LEAFED NON-BROWNISH-FLOWERED	YELLOW-LEAFED BROWNISH-FLOWERED	TOTAL
1	30	18	12	0	60
2	7	3	1	0	11
3	42	11	20	0	73
4	9	2	3	0	14
5	15	7	7	0	29
Total	103	41	43	0	187

reference to the genotypic constitution of the double-dominant F_2 plants, we should expect, theoretically, 20,002 cases of dihybrid segregation (of which 20,000 would segregate in repulsion phases, while 2 would represent the coupling segregation), 400 cases of monohybrid segregation, and only 1 case of constant family, in every 20,403 trials. In view of this consideration it would be natural to expect only repulsion segregations when only five families are examined.

MIYAZAWA (1918) obtained a number of the double recessives from one of the green-leafed and brownish-flowered F_3 plants. Such a segregation was quite unexpected on the basis of his hypothesis of factor interrelation.[7] According to the linkage hypothesis, on the contrary, the case is easily explained. The origin of this family should be considered as starting from the union of a non-crossover gamete, *gr*, and a crossover one, *Gr*.

SUMMARY

1. About 1.09 percent of crossing over occurred in the segregation of dihybrid crosses involving the *contracta* type of foliage and the white-margined flower in the Japanese morning-glory (*Pharbitis nil*).

2. Nearly the same percentage (1.01 percent) of crossing over was also found in the linked characters, yellow leaf and brownish flower.

3. MIYAZAWA'S (1918, 1921) hypothesis of "factor interrelation" applied to the inheritance of yellow leaf and brownish flower is refuted.

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[7] In his first paper MIYAZAWA (1918, p. 73) notes as follows:

". . . ., but here some yellow plants with dark-red flowers made their appearance, which were never found otherwise. This peculiar case will be the subject of my future paper."

On this point he writes in his second report (MIYAZAWA 1921, p. 10) as follows:

". . . . it may be considered that there occurred some permanent variation among the factor or factors in the F_3 plant used for self-fertilization. But we cannot decide how and where such change has occurred, and any hypothesis on this point would be useless unless founded on the facts actually obtained, so that this case will remain the subject of my future study."

THE REGIONALLY DIFFERENTIAL EFFECT OF X RAYS ON CROSSING OVER IN AUTOSOMES OF DROSOPHILA[1]

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THE PROBLEM

It has been stated by MAVOR (1924) that "We see in the effects which X rays produce on crossing over in the first and second chromosomes of Drosophila a remarkable instance of opposite effects. . . . ." This statement was based on extensive and critical data, which showed clearly that there was a significant decrease in crossing-over frequency between white and miniature, in the first chromosome, but a significant increase between black, purple and curved, in the second chromosome, after application of similar doses of X rays to both. MAVOR (1924) further noted that in the case of the black-purple and purple-curved regions, "the greatest effect for the same dose is produced in the black-to-purple region,"—and, as calculations to be reported in the present paper now prove, the differences between the effects observed by MAVOR on these two regions of the second chromosome are statistically significant. This observation, however, raises the question whether crossing over in still other regions of the same chromosome (II) might not show even greater differences in the effects of X rays than those shown between the black-purple and purple-curved regions. If so, a comparison could scarcely be drawn between the

[1] Contribution No. 193 from the Department of Zoölogy, of the UNIVERSITY OF TEXAS.

effects of X rays on different chromosomes as a whole, inasmuch as the crossing-over reactions would be highly specific for individual chromatin regions considerably smaller than those blocks of chromatin comprising the morphological chromosomes.

An investigation concerned with the above problem,—i.e., designed to reveal possible intrachromosomal differential effects of the X rays on crossing over,—had been begun by the present writer before the suggestive results on black-purple-curved, quoted above, came to hand, for certain already-existing data had made the finding of such differences not unlikely. The reasons for suspecting such effects may be listed under two heads, cytological and genetic.

In regard to the cytological evidence, it is to be noted that the central regions of the long V-shaped autosomes (chromosomes II and III) are morphologically different from the distal arms of the same chromosomes in at least four respects: (1) The spindle fibre is attached in the central regions, and not in the distal; (2) The bend of the chromosome regularly occurs in the central regions; (3) The chromatin appears to be less deeply staining there; (4) It is narrower in this vicinity.

The purely genetic grounds for expecting regionally differential chromosome reactions are as follows: (1) PLOUGH (1917, 1921) had shown that extremes of temperature affected crossing over in the central regions of both the long autosomes, but not in any of the distal regions which he studied; the latter behaved like the greater portion of the rod-like X chromosome in this respect. (2) BRIDGES (BRIDGES 1915; BRIDGES and MORGAN 1919, 1923) had made similar findings with regard to the effect of age on crossing over. (3) BRIDGES and the present author had noted that the central regions of the autosomes have a higher coincidence of crossing over than the distal regions,—which in this feature also resemble the X chromosome more closely. (4) The standard maps, based on the collective Drosophila work (given in BRIDGES and MORGAN 1919 and 1923) show a tendency to a concentration of mutant genes in the central regions of both chromosomes. (5) The same effect appears decisively in the maps of lethal genes in chromosome II found in the present writer's mutation studies (MULLER 1920). This apparent crowding effect in the middle regions might be due either to mutations occurring more frequently there than in the distal regions, or to a relatively lower frequency of crossing over in the middle regions. Of these two possible explanations the latter is by far the more probable.

All told, the above facts furnish a considerable body of evidence which demonstrates that the crossing-over reactions of the chromosomes

throughout their length cannot be studied through data based on limited regions of them. And it is evident, specifically, that the central regions of the long autosomes have crossing-over properties which distinguish them especially from other portions of the chromatin. Now, it so happens that the loci of black, purple and curved, used by MAVOR, all lie near the middle of the second chromosome. Results based on these loci, therefore, must be regarded as particularly unrepresentative of the reactions of the rest of this chromosome, and before we can judge of the crossing-over reactions to X rays of this chromosome, in general, in comparison with reactions of other chromosomes, it is necessary to study distal as well as central regions.

Experiments directed expressly towards a study of the differential effect of X rays on the different regions within the large V-shaped autosomes were carried out with both the second and third chromosomes. It was necessary, in this work, to follow the distribution of a considerable number of loci, scattered throughout the major portion of the lengths of these chromosomes in such a way as to include and differentiate between the middle and other regions, and extending distally to one end at least. It was preferable too that all the loci studied in a given chromosome should be followed simultaneously, so that differences in crossing-over frequency due to uncontrolled factors that might vary from experiment to experiment would be avoided. Such an arrangement would, in addition, permit a more accurate study of coincidences of crossing over, and of the effect of X rays upon the coincidences involving various regions.

It was also of interest to determine whether or not chromosomes otherwise identical might respond differently to X rays according to whether other factors affecting crossing over,—such as, for instance, specific genes hindering crossing over,—were different. Hence, a variation was introduced into one of the experiments by using a chromosome containing one of these genes.

Finally, it was thought desirable to vary to some extent the X-ray dosage employed, so that possible variations in the effect on different regions due to this cause might be observed.

TECHNIQUE

A multiple-recessive stock was used for the second-chromosome study, containing the six mutant genes dumpy (T^d), black (b), purple (p_r), curved (c), plexus (p_z) and speck (s_p). This stock, which had been made up chiefly by BRIDGES, and to which T^d had been added by the author, may be referred to here as "II-ple." The plexus character was not followed

in the counts. The positions of these loci in the standard map (BRIDGES 1921, unpublished) are shown in figure 6. It will be seen that the first gene is 8 units from the "left-hand" end and the other genes scattered over the remainder of the chromosome all the way to the extreme "right-hand" end. In the mid-region, which requires more minute study, the genes are more closely spaced.

For the third chromosome a multiple-recessive stock designated as "III-ple" was used. This stock, made up chiefly by BRIDGES, contains roughoid (r_u), hairy (h), scarlet (s_t), pink (p), spineless (s_s) and ebony (e). The positions of these genes on the standard map (BRIDGES and MORGAN 1923) are shown in figures 4 and 5; the first gene is at the extreme left-hand end and the others scattered, up to about 30 units from the right-hand end. Here, too, there is closer spacing in the middle and a rather wide even spacing elsewhere. In the case of both stocks the loci closely on either side of the mid-point (b and p_r, and s_t and p, respectively) are so located as to include a larger section of chromatin to the left than to the right of the center, and there are other loci (c and s_s, respectively) bounding a region (p_r-c and p-s_s) somewhat farther to the right of the middle, yet appreciably closer to the latter than is the region (T^d-b and h-s_t, respectively) to the left of the most central region. Farthest of all from the center, in each case, are the remaining regions, c-s_p in II and s_s-e and r_u-h in III, the first two named being to the right, the last to the left of the center. Hence, in each case there are several regions for study, increasingly distant from the center, and at distances from it that are comparable in the two stocks.

In the first experiments, in order further to make the data comparable, flies of as nearly identical composition as practicable were secured for the studies on the two chromosomes. This was effected by crossing flies from the II-ple stock to III-ple flies (heterozygous for the second-chromosome gene curly, C_y, which will be referred to later) and using the female offspring, heterozygous for both sets of genes, for either study, in the one case by backcrossing them to II-ple males, and in the other case by backcrossing to III-ple males. Identical food and environic conditions were used, of course, in all cases. The controls and the individuals to be X-rayed, in the case of the flies of both second- and third-chromosome experiments, were gotten by subdividing the latter groups of flies by random selection, and the control and X-ray lots so obtained were in each case subjected to identical conditions, apart from the raying. As flies from the same bottle, all of similar age, were employed throughout, and (after separation into curly and non-curly) divided at random between

the treated and control lots, it will be seen that the flies in any of the treated lots were genetically and developmentally as much like those in the corresponding control as they were like each other.

Both control and X-ray lots of flies were kept virgin until the time of raying, when all were between one and two weeks old. After the raying they were mated and kept in a first set of bottles for six days, and then transferred to other bottles. The offspring from the first bottles were discarded, inasmuch as MAVOR has shown that the effect of raying on crossing over does not commence immediately, and it was desired to secure results which would be homogeneous. Each female was placed, with one male, in a separate bottle, when the cultures that were to have counts made upon them were made up, and separate records were kept of the results from each pair. The offspring counted were derived from the eggs laid from the sixth to the twelfth day after raying.

On the female flies referred to above, derived from the cross of II-ple by III-ple, heterozygous for curly, three sets of experiments were conducted. In experiment "a," one lot, consisting of 14 curly females (not counting those that proved sterile) were backcrossed to III-ple males, for a study of crossing over in the third chromosome. Seven of these were rayed and seven served as controls. (The non-curly flies were not used in this case, because the gene for black, present in the latter heterozygously might have tended to confuse the classification of the offspring in regard to ebony). In experiment "b" another lot, consisting of 18 (fertile) non-curly females were backcrossed to II-ple, for a study of crossing over in the second chromosome; 10 of these were rayed. In experiment "c" a third lot, consisting of 15 (fertile) curly females, were backcrossed to II-ple, and of these 5 were rayed. In this third lot an inhibition of crossing over was expected to occur, owing to the presence of curly, which has been found by WARD (1923) greatly to reduce the frequency of crossing over in the second chromosome,—without, however, affecting it in the third chromosome.

A much weaker dose of X rays was applied than that used by MAVOR, in order not to affect fertility adversely and also to bring to light any difference in effect from that observed by him, which might be produced by a lesser dose of the rays. PLOUGH (1924) has recently suggested that smaller doses may have an effect on crossing over opposite to that produced by larger doses, and that a smaller dose, in the case of a chromosome more sensitive to the rays (as he suggests that chromosome II may be) would then produce a similar effect (decrease of crossing over) to that observed in the case of a less sensitive chromosome (the X chromosome)

when a large dose is applied. If such a relation held, then, it might be revealed when a small dose of X rays was applied.

The dose used in the experiments here referred to may be designated as 26.8 H (Holzknecht units). One H, or Holzknecht unit, may be described as the amount of radiant energy received from unfiltered rays resulting from a current of 3 milliamperes, at 30,000 volts, when applied for 1 minute at a distance of 20.3 cm (8 inches). The radiant energy received, in number of H, may then be calculated by the formula: $\frac{\text{volts} \times \text{milliamperes} \times \text{minutes}}{(\text{centimeters})^2} \times .00458$. (One H is then equal to .437 of MAVOR's "D," when 50,000 volts are used.) In the present work unfiltered rays from a broad-focus Coolidge tube with tungsten target were employed throughout, and the voltage was kept at 50,000. In experiments a, b and c, the flies of which were treated simultaneously, the number of milliamperes averaged 4, the time 7.5 minutes, and the distance 16 cm. The total energy absorbed by the tissue in question, rather than these individual items, has been shown usually to be the more significant factor in determining the degree of various biological effects of X rays, particularly if the spectrum is constant, and MAVOR's work has verified the truth of this rule with regard to the effect on crossing over, specifically. H gives, of course, the amount received rather than absorbed, but since, with a given spectrum and a given tissue, the proportion of the received rays that are absorbed is constant, the dosage under circumstances like those in the present experiment may be expressed in H. The dose of 26.8 H used here was little more than half of the minimum dose, 48 H (21 "D"), used by MAVOR in his crossing-over work, and slightly less than a third of the dose designated as optimal by him, namely 80 to 86.8 H (35 to 38 "D").

It will be seen later that when the results of the above experiments were tabulated, some apparent differences between the results on both second and third chromosomes and the comparable portion of MAVOR's work on the second chromosome appeared. In order to obtain evidence as to whether these differences were really due to the difference in X-ray dosage, and also in order to secure, if possible, more marked effects on the third chromosome, with a dose similar to that which MAVOR had used on the second chromosome, the part of the experiment relating to chromosome III was then repeated (experiment "d"), with the larger dose of 54.4 H. This dose is somewhat larger than MAVOR's minimum dose and twice as large as the dose previously used by the present writer. The cross was made and the entire experiment was conducted as before, with the

following exceptions: (1) A different stock of II-ple was used, not containing curly; consequently, non-curly female offspring, heterozygous for

A

Culture No.												region of c. o.
Date of Count												
Normals												0
h st p ss e												1
st p ss e												2
p ss e												3
ss e												4
e												5
ru e												1,5
ru ss e												1,4
ru p ss e												1,3
ru st p ss e												1,2
ru												1
ru h st p ss e												0
Aberrant												
Totals												

FIGURES 1, 2 and 3.—Tabulation forms for complex linkage counts (backcross of $\frac{r_u\, h\, s_t\, p\, s_s\, e}{+}$) Directions for fitting forms together are given in the text.

II-ple and III-ple, were bred, being backcrossed to the III-ple males. Fourteen females were treated, and eight kept as controls. (2) The females

were three weeks old when bred. (3) The offspring hatched resulted from eggs laid during a period of four to twelve days after raying. (4) Two females were bred in each bottle. Owing to the first two differences the crossing-over frequencies in the controls of this experiment were significantly different from those in the controls of the preceding experiment.

In the account that follows, the results of the experiments will be reported in the order a, d, b, c, as this sequence is better adapted for a comparison of significant features.

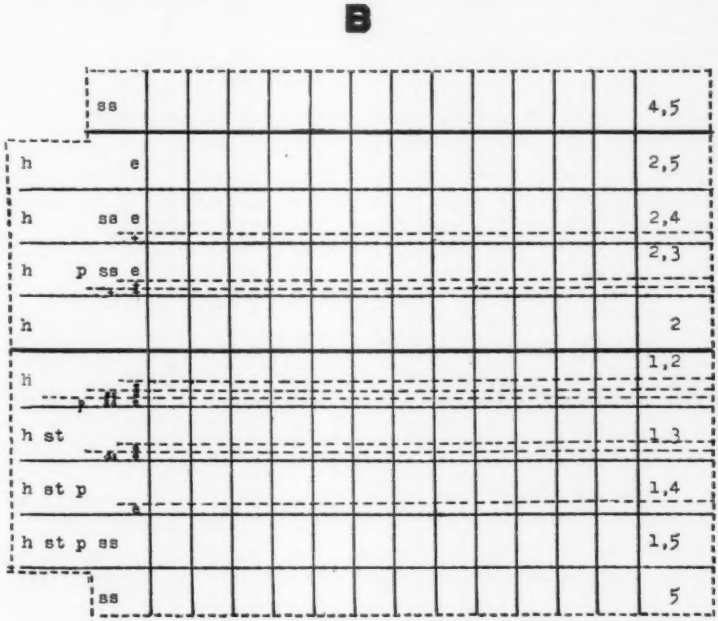

Fig. 2

In recording counts like those made here, which involved either five or six, usually six, pairs of characters, and 32 or 64 possible combinations of characters, the tabulation is ordinarily very cumbersome and time-consuming,—a fact which seriously limits the numbers which it is feasible to count. An effort was therefore made to devise a more convenient system of tabulation, and a scheme was arrived at which proved so useful that it may be given here (figures 1 to 3) for the benefit of any workers who have similarly complicated linkage counts. The scheme as here

presented is for the recording of 6-locus third-chromosome counts, from females having III-ple in one third chromosome and all normal genes in the other, but it can of course be adapted, by changing and rearranging letters, to any other 6-locus count, and a similar scheme may be used for any number of loci.

The tabulating form is made by cutting out the three pieces along their outer dotted lines, and folding along the heavy lines, as follows. Each of the two smaller pieces (B and C) is first folded back along its mid-line

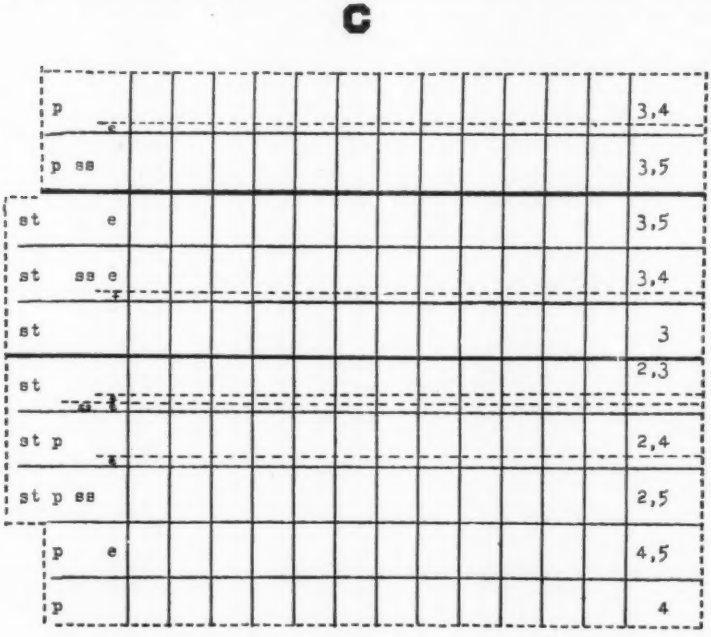

Fig. 3

and its two apposed halves are pasted, or held together with labels. Each (doubled) piece is then held with that side upward on which the remaining heavy line appears, and so placed that the letters seen are upright. The piece is then folded over towards the manipulator along this remaining heavy line, by leaving the lower, larger section of the piece in place and inverting the smaller, upper section over it, so that the back of the latter appears in view (with its letters now in upright position). This fold is not pasted together but left as a movable hinge. Piece B is

then attached to A along its movable hinge by means of labels, in such a position that the hinge lies along the central line of A, and identical letters of the two pieces lie in the same vertical line. Similarly, piece C is fitted into piece B, within its movable fold, so that its hinge lies along the same line as that of B and its identical letters are in line with those of A and B. When B and C are both in their folded, or more usual position, C then lies attached within the fold of B.

The advantage of this form lies in the fact that the combinations most frequently encountered—non-crossovers and single crossovers—are compactly and systematically arranged in open view, for ready recording of results, and in addition the other classes are not scattered along so that a search is necessary for finding them, but are immediately exposed by the left hand when the finger is inserted in the space between the letters representing the first two genes between which crossing over has occurred. Thus, if a double crossover commencing r_u h non-s_t is found (this involves a crossing over first between h and s_t) the finger is inserted upon r_u h, so as to remove s_t, by folding upwards the sheet containing this symbol. This brings to view, at the point of the finger, the space for recording such double crossovers. On the other hand, if the double crossover had been non-r_u non-h s_t, the finger, isolating s_t from r_u h, and following it upwards, points to the record of all double crossovers commencing in s_t. If one thinks of the characters in the order in which their corresponding genes lie in the chromosome, the finding of each given combination on the table by the above method soon becomes practically automatic. The convenience of the form of tabulation here described, and the ease with which a given class is found by means of it, is, however, more readily appreciated after a little manipulation of the form than by long explanations. It will likewise be found that this form saves much time when the results are treated for analysis, as the employment of the third dimension makes possible a more systematic juxtaposition of corresponding classes, and a readier summation or comparison of them than can be secured in the ordinary table. Forms of this type, once worked out, may be mimeographed, and are then available for extensive counts.

In taking the record, it saves time first to pick out the flies of the classes expected to be most numerous (the non-crossovers), to count and record these and get them out of the way, then to proceed to the next most numerous, and so on down, for this results in looking over the same fly the smallest number of times. Another way is simply to keep tally, by classifying each fly in succession as it comes and recording this with a mark in the appropriate square of the table. In that case the time-saving

feature of the present as compared with other forms of tabulation becomes most marked, but tallying is not advisable unless the proportion of classes to number of flies counted is very large.

The writer is deeply indebted to Doctor DALTON RICHARDSON, Roentgenologist of Austin, Texas, for the generous spirit of scientific coöperation in which the latter carried out the actual treatment of the flies, with his own apparatus.

In addition, the writer wishes to acknowledge the efficient and conscientious aid given him by his students, Mr. G. LANGNER, Mr. H. LEFKOWITZ, Miss A. RUYSENAARS and Miss F. SETTLES, in the making of the counts in the present experiments involving the lighter dose.

DATA ON REACTION OF THIRD CHROMOSOME TO LIGHTER DOSE

In table 1, data from the experiment on the third chromosome, involving the series treated with a dose of 26.8 H (11.7 D), and its control, are presented in condensed and analytic form, the percent of crossing over, or "distance," between each two neighboring genes being given, and also

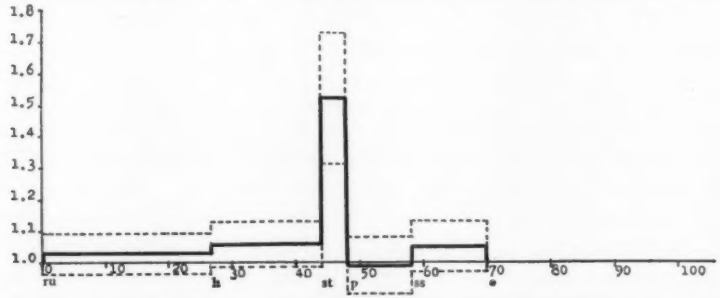

FIGURE 4.—Effect of lighter dose on chromosome III. Heavy line represents quotient of treated divided by control crossover value, height of line (ordinates) giving size of quotient for region of chromosome shown on base line (abscissae). Upper and lower dotted lines give values of quotient plus and minus its probable error. Points on base line are plotted according to the standard map values.

the total percent of crossing over, or "map length," and the total number of flies counted. After each of the percents is given its "probable error" (to which it would be subject if random samples of the given size were taken out of an indefinitely large lot of individuals having the given percent as its true percent). The values for the treated series are given on the first line, those for the controls on the second line. The differences between percents for treated and control series, with their "probable errors" (taken on a basis corresponding to that above) are given on the

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TABLE 1

Effect of lighter dose on crossing-over frequency in chromosome III.

ORIGIN OF DATA	PERCENT OF CROSSINGS OVER IN:						NUMBER COUNTED
	Region 1 (r_w–h)	Region 2 (h–s_t)	Region 3 (s_t–p)	Region 4 (p–s_s)	Region 5 (s_c–c)	Total distance (r_w–c)	
Treated series, "T"	25.1±1.19	22.1±1.14	7.3±.71	12.4±.90	17.2±1.03	84.1±1.86	605
Control series, "C"	24.3±.97	20.8±.90	4.8±.48	12.5±.74	16.4±.82	78.8±1.73	917
Difference, T−C	+1.8±1.54	+1.3±1.45	+2.5±.87	−0.1±1.17	+0.8±1.32	+5.3±2.5	
Difference÷ probable error	1.2	0.9	2.9	0.09	0.61	2.1	
Quotient, T÷C	1.03±.064	1.06±.071	1.52±.212	.99±.093	1.05±.082	1.07±.033	

third line, and the number representing each difference divided by its own probable error is recorded on the fourth line. On the fifth line the ratios, or quotients, formed by dividing the percent for the treated by that for the control series, are given, with their probable errors (also on a corresponding basis); a graphic representation of the quotients and their probable errors is shown in figure 4.

Examination of the table shows at once that the only region in which a difference at all significant occurred between treated and control series was s_t-p, or region 3, the section which is believed to include the middle point of the chromosome (at which the spindle fibre becomes attached). The differences in all the other regions were scarcely, if any, greater than their own probable errors, but in this middle region the difference was of a size (2.9 times its probable error) such that it would have occurred only once in twenty times had the treated and control really had the same crossing-over value. The difference was, moreover, in the same direction as that found by MAVOR for the central region of the other long autosome, —that is, an excess of crossing over in the treated series. It is, therefore, probable (with a probability of 19 to 1, according to the usual method of reckoning) that a dose of X rays of only about 27 H increases the frequency of crossing over in the center of the third chromosome. There is no evidence, however, that this dose of X rays affects crossing over elsewhere in the third chromosome, either by increasing or decreasing it, and it is noteworthy that this statement applies to p-s_s, the region next nearest the center, at least as strongly as to the regions further removed, whereas in MAVOR's work on the second chromosome, in which a larger dose was used, the region, p_r-c, corresponding to p-s_s, was affected decidedly, and in the same sense as the most central region.

Although the ratios of treated to controls are, of course, subject to considerable error, they are a measure of the intensity of the effect produced by the treatment,—so far as this may be revealed by the data at all, —and, as will be seen later, it will be desirable to compare these ratios statistically both with each other and with the ratios obtained in the other experiments.

The total map lengths of the chromosome sections studied show an excess of 5.3 units in the treated series. Although such a difference (2.1 times its probable error) should occur, on the average, once in every six samples of the given sizes even if the true map lengths were the same, nevertheless, it may be taken as slight evidence in favor of the total map length being increased by treatment. The increase found, however, gave the treated series a length of only 1.07 ± 0.3 times that of the controls,

TABLE 2

Effect of lighter dose on coincidence in chromosome III.

ORIGIN OF DATA	REGIONS INVOLVED:					
	1 and 2	2 and 3	3 and 4	4 and 5	2 and 4	1 and 5
Treated series, "T".....	0.57±.077	1.1±.205	2.4±.358	0.31±.104	1.2±.174	1.09±.133
Control series, "C".....	0.60±.068	1.4±.242	1.1±.280	0.37±.085	0.8±.115	1.26±.10
Difference, T−C.....	−0.03±.102	−0.3±.32	+1.3±.45	−0.06±.135	+0.4±.21	−0.17±.166
Difference÷probable error.....	0.29	0.94	2.9	0.44	1.9	1.02

showing that the chromosome as a whole was, at any rate, not much affected.

The percents of crossing over, considered in each region, separately, are not the only values about which it is desirable to obtain information. The data also contain information regarding the amount of coincidence of crossing over, that is, the frequency of double crossovers, as compared with the proportion of double crossovers which would have occurred if crossings over in the two regions considered had been independent of one another (see MULLER 1916, WEINSTEIN 1918). The values of this "coincidence," for each two contiguous regions, and some others, are given in table 2. In the case of both treated and controls the coincidences between adjacent regions (involving distances of 0 to 30 or 40 for the double crossovers involved) are significantly below 1.0 (the expectation if crossings over in the two regions were independent) in the outer parts of the chromosome, but within the range of error of 1.0 in most cases involving even short distances in the middle of the chromosome. This result is in accord with those of previous experiments on the (non-X-rayed) third as well as second chromosomes. One coincidence, however, that for 3,4,—the middle and an adjacent region,—in the treated series seems significantly higher than 1.0. Much stress cannot be laid upon this fact, as the unavoidable rare errors which may be made in classification necessarily affect the accuracy of the small double-crossover classes far more than that of the other classes. The difference between the 3,4 coincidence values of treated and control is 2.9 times its probable error. As this occurs in the very region in which crossing over has been increased, it may represent a real difference. On the whole, however, the coincidences for adjacent regions are strikingly alike in treated and control series.

If the coincidences for adjacent regions approach 1.0, it might be expected that those for more distant regions would be still more likely to show this ratio connoting independence of crossings over. The coincidences of distant regions might, therefore, have less chance to show an effect from the treatment than those of adjacent regions. Those for 2,4,—the regions on either side of the center,—were calculated, as being most likely to show an effect from treatment, but, as the table shows, they do not depart significantly from 1.0 or from each other. Coincidences for 1,5 were also calculated,—as representing the most widely separated regions,—but here too they are within range of 1.0 and of each other.

In calculating these coincidences for regions not adjacent to each other, only that part of the data was allowed to enter into any of the elements of the computation, which comprised individuals not having a crossing over

TABLE 3

Effect of heavier dose on crossing-over frequency in chromosome III.

ORIGIN OF DATA	PERCENT OF CROSSINGS OVER IN:						NUMBER COUNTED
	Region 1 (r_w–h)	Region 2 (h–s_t)	Region 3 (s_t–p)	Region 4 (p–s_a)	Region 5 (s_a–e)	Total distance (r_w–e)	
Treated series, "T"	20.8±.67	20.0±.66	6.2±.40	10.7±.52	10.8±.52	68.5±1.19	1638
Control series, "C"	23.3±1.0	17.8±.89	3.1±.40	5.9±.55	11.4±.74	61.5±1.51	847
Difference, T−C	−2.5±1.2	+2.2±1.11	+3.1±.56	+4.8±.76	−0.6±.90	+7.0±1.92	
Difference ÷ probable error	2.08	2.0	5.5	6.3	0.7	3.65	
Quotient, T÷C	0.89±.048	1.12±.067	2.0±.289	1.8±.189	0.95±.076	1.11±.033	

in an intermediate region. The resultant coincidence coefficient, which may be called a "partial coincidence," shows more correctly the possible influences upon one another of crossings over in the two regions considered, than the ordinary coincidence coefficient, which entails the disturbing influence of intermediate crossings over, would do. The effect of the intermediate crossing over, being to "inhibit" crossing over both to left and right of it, causes a reduction in the number of crossings over that happen simultaneously in those two regions, and thus may cause an apparent negative correlation (reduction of coincidence below 1.0) between these distant crossings over, even though they may be having no direct

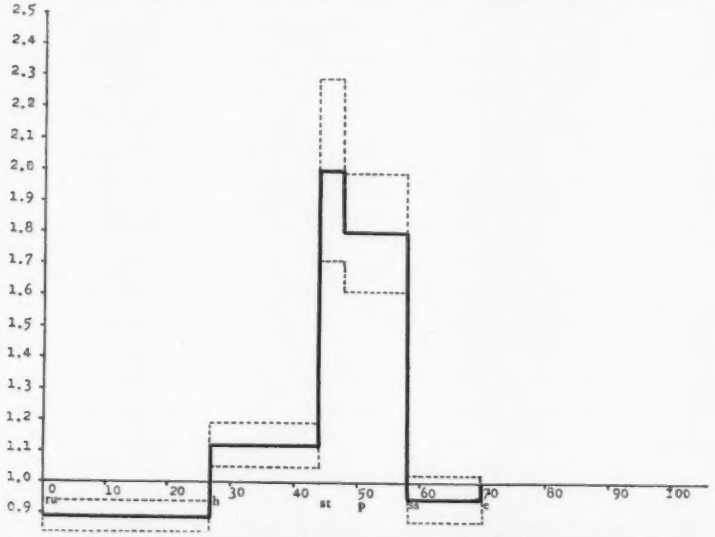

FIGURE 5.—Effect of heavier dose on chromosome III. Details as in figure 4.

influence on each other. The "partial-coincidence" coefficient, avoiding this source of error, has in our own experiments shown a coincidence within the range of error of 1.0 for the more distant regions in at least fourteen out of sixteen cases, whereas the ordinary coincidence would in most of these cases have been below 1.0.

DATA ON REACTION OF THIRD CHROMOSOME TO HEAVIER DOSE

The results obtained for crossing-over frequency in the third chromosome when the dose of X rays was doubled, and those of the control series bred simultaneously, are shown in table 3, in which the data are presented

in the same manner as in table 1, already described. Figure 5 is a graphic representation of the quotients of table 3, and their probable errors.

It will be seen that in this experiment, both the most nearly central region, s_t-p, and that next nearest to the center, p-s_s, showed a highly significant excess of crossovers in the treated as compared with the control counts. The chance that such differences should have been found in random samples of the same material is considerably less than 1 in 5000, in each case. On the other hand, the differences in the other regions, which, taken at their face values, suggest a slight decrease of crossing-over frequency in the more distal regions of the chromosome, have no distinct statistical significance.

The result, then, seems to prove that the effect of X rays in increasing crossing over, is, with larger doses, not confined to the very center of the chromosome. Hence, the effect could not be due to a mere increase in the number of breaks at a given point—such as the point of attachment of the spindle fibre. This result for the third chromosome is in accordance with the results obtained by MAVOR, with similar doses of X rays, for the second chromosome, inasmuch as he found both the central region (b-p_r) and one adjacent to the center (p_r-c) to be affected; in his work, however, as no other regions were studied, the question of a possible localization of the effect was not raised. In the present experiment there is also some slight evidence of an increase of crossing over in the region (h-s_t) adjacent to the center on the left, but the increase indicated is small, in accordance with the fact that this region averages considerably farther from the center than does the adjacent region on the right (p-s_s). The total increase indicated, for all the central regions, is 10.1 units with this dose, as compared with 3.7 units in the case of the dose half as large.

The total map length in the present experiment is 7 units greater in the treated than in the control series, a difference having distinct statistical significance (chance in random sampling 1 in 250), but somewhat smaller than the difference in the more central regions taken by themselves, owing to the slight decreases found in the distal regions. It may be noted here also that, owing to the fact that most of the coincidences remained approximately 1.0 in the treated series, this increase in map length did not carry with it any significant change in the percent of actual separations between the two most distant points considered, r_u and e, even when this relatively strong dose of X rays was employed; for the percent was 44.5 ± 1.2 in the controls and 43.9 ± 0.8 in the treated counts,—actually a (non-significant) decrease of 0.6 percent $\pm$ 1.42 percent in treated as compared with controls.

TABLE 4

Effect of heavier dose on coincidence in chromosome III.

	REGIONS INVOLVED				
	1 and 2	2 and 3	3 and 4	4 and 5	1 and 3
Treated series, "T"	0.37±.046	1.1±.126	1.0±.192	0.42±.095	0.88±.122
Control series, "C"	0.28±.056	0.22±.143	2.0±.712	0.18±.12	0.75±.202
Difference, T−C	+0.09±.073	+0.88±.19	−1.0±.74	+0.24±.153	+0.13±.24
Difference÷probable error	1.2	4.6	1.35	1.6	0.56

TABLE 4 (continued)

	REGIONS INVOLVED				
	2 and 4	3 and 5	1 and 4	2 and 5	1 and 5
Treated series, "T"	1.05±.10	0.86±.177	1.03±.10	1.02±.101	1.07±.111
Control series, "C"	0.70±.176	0.45±.174	1.44±.186	1.11±.141	0.625±.109
Difference, T−C	+0.35±.203	+0.41±.25	−0.41±.212	−0.09±.174	+0.445±.156
Difference÷probable error	1.72	1.61	1.93	0.52	2.85

The coincidences, which were all calculated, are shown in table 4, together with their probable errors, and the differences between treated and control coincidences, with their errors, and the number of times these differences are of their errors. In the case of non-adjacent regions the partial coincidences were obtained as before, instead of the gross coincidences, by elimination from the data of all crossovers occurring in regions lying between those considered.

It will be seen that the values obtained here are, on the whole, very similar to those of the preceding experiment, being low, distinctly below 1.0, for nearby regions in the distal parts of the chromosome, but within range of 1.0 in the great majority of the other cases. In all but two of the 10 pairs of cases, moreover, the values are substantially the same in treated and control series, the difference being not more than twice its probable error. One of these pairs of aberrant cases is coincidence 2,3, involving the middle and an adjacent region. This suggests, as did the aberrant difference for coincidence 3,4 in the first experiment, that in the central region the coincidence is being increased by X rays. Neither experiment really corroborates the other in regard to this question, however, since the conspicuous difference in each experiment is balanced by an equality of the exactly corresponding coincidences in the other experiment. Comparison of the controls and treated in the two experiments suggests that the difference between control and treated values of coincidence 2,3 in the present experiment may really be due to an abnormally low value for this coincidence in the present control, as the treated value of the present experiment is about the same as both treated and control values in the other experiment. The same consideration holds with regard to coincidences 1,5, in which the treated value in the present experiment conforms to the 1.0 value to be expected for such distant regions, and to the value obtained in both treated and control in the other series. After all, however, the difference between treated and control coincidences 1,5 in the present experiment does not represent a very rare chance for samples from the same material, being less than 3 times its probable error. And, as explained previously, coincidence values are likely to be subject to other errors than those of random sampling in greater degree than are crossing-over frequency values.

On the whole, then, the similarity of the coincidence values obtained in treated and controls, and of the values obtained in the two experiments, is the most striking feature of the coincidence results. The study does not include, however, regions near the center which are small enough and close enough together to have coincidence values which are normally

TABLE 5

Effect of lighter dose on crossing-over frequency in chromosome II.

ORIGIN OF DATA	PERCENT OF CROSSINGS OVER IN:					NUMBER COUNTED
	Region 1 (T^d–b)	Region 2 (b–p_r)	Region 3 (p_r–c)	Region 4 (c–s_p)	Total distance (T^d–s_p)	
Treated series, "T"	23.2±.64	8.73±.44	19.4±.61	26.8±.67	78.13±1.37	1969
Control series, "C"	21.6±.72	6.26±.42	17.9±.67	26.0±.75	71.76±1.39	1502
Difference, T−C	+1.6±.94	+2.47±.62	+1.5±.91	+0.8±1.0	+6.37±1.95	
Difference÷probable error	1.7	4.0	1.65	0.8	3.27	
Quotient, T÷C	1.07±.046	1.4±.12	1.08±.053	1.03±.04	1.09±.028	

distinctly below 1.0, and so it cannot be concluded that X rays would have no effect upon coincidences of small magnitude in this portion of the chromosome. But it can be concluded that coincidences of small magnitude in distal regions, and also all coincidences normally approximating 1.0, in any regions, are affected little or not at all by these doses of X rays.

DATA ON REACTION OF SECOND CHROMOSOME TO LIGHTER DOSE

The data relating to crossing-over frequency in this experiment have been analytically presented in table 5 and figure 6 in similar fashion to those of the preceding experiments. A glance at the figure or at the table, (especially at the line of quotients, which is the most informative) shows the remarkable similarity between the results here and in the experiment on the third chromosome in which the same dose was used. In the present experiment, as in the other, the only region distinctly affected was the centermost region (here b-p_r), in spite of the fact that MAVOR, with his larger dose, found that the other region studied by him, p_r-c, was affected

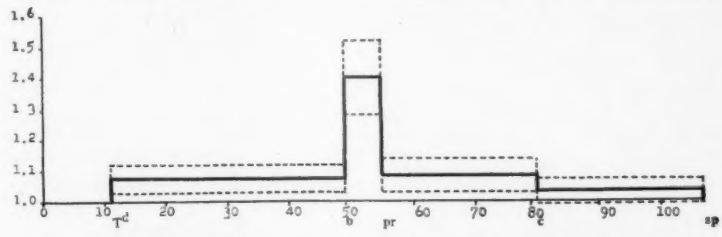

FIGURE 6.—Effect of lighter dose on chromosome II. Details as in figures 4 and 5.

also. A difference as large as that in the centermost region in this experiment would arise less often than once in 140 trials, if due purely to chance; differences as large as those in any of the other regions would arise oftener than once in 4 trials. The increase in the length of the total map,—6.4 units,—is also fairly significant, and about the same in amount as the increase (5.3) in the map length of chromosome III under the same conditions.

The coincidences for adjacent regions were calculated, and are given in table 6. As in the case of the corresponding coincidences in chromosome III, these are significantly lower than 1.0 for regions chiefly on the same side of the midpoint, but are approximately 1.0 for regions chiefly on opposite sides of the mid-point, even when these regions are adjacent and fairly short (2 and 3). This result agrees also with previous data on coincidence in chromosome II. Of greater interest is the fact that, as was

found to be the usual rule in chromosome III, there are no significant differences between the coincidences for adjacent regions in treated and control lots. (Coincidences for non-adjacent regions here were all within close range of 1.0, since in these cases the regions were always on opposite sides of the mid-point.) Again we must not conclude from this correspondence that in the central portion of the chromosome the smaller coincidences which might have been obtained from regions still closer together would not have shown significant differences.

The close parallel between the results for the second and the third chromosomes, when treated with the lighter dose, makes more significant the increase in crossing-over frequency in the central region of chromosome III, which by itself would not have seemed so decisive. Further, the marked difference between the reaction to the weaker and that to the

TABLE 6

Effect of lighter dose on coincidence in chromosome II.

ORIGIN OF DATA	REGIONS INVOLVED		
	1 and 2	2 and 3	3 and 4
Treated series, "T"	0.6±.076	1.2±.106	0.6±.049
Control series, "C"	0.8±.115	1.0±.13	0.7±.055
Difference, T−C	−0.2±.138	+0.2±.168	−0.1±.074
Difference÷probable error	1.45	1.2	1.36

stronger dose shown by the region next in proximity to the center acquires much greater significance from the fact that this same phenomenon appears both in the third chromosome (as previously noted) and in the second (as shown by comparison of the present results on p–c with those of MAVOR). The relative lack of effect of either dose on the frequency of crossing over in the distal regions is also made more certain by the correspondence of all the present experiments in this respect. That this apparent lack of effect, in these long regions, cannot be due to a relative increase in the proportion of double crossovers is proved by the coincidence studies.

DATA ON REACTION OF SECOND CHROMOSOME CONTAINING CROSSOVER-INHIBITORS TO LIGHTER DOSE

The chromosome bearing the gene for curly wings contained the crossover-inhibiting genes studied by WARD (1923),—one located in the left

half and preventing nearly all crossing over in this section, and the other in the right half, preventing most of the crossing over there. These effects occur in flies heterozygous for the genes (homozygous curly is lethal). The central region of the chromosome is less affected by either of these genes than the distal region in which the gene is located; thus even when both genes are present there is a small amount of crossing over between *b* and p_r, and between p_r and *c*. This is illustrated in the controls of the present experiment, on the second line of table 7, where it is shown that 2 crossovers were found in the first and 1 in the second of these regions, but none elsewhere, amongst the 2060 flies. Amongst the 901 flies from treated parents there were 9 crossovers altogether, 5 of these being in the most central and 4 in distal regions. Although the total numbers of

TABLE 7

Effect of lighter dose on chromosome II with curly present.

ORIGIN OF DATA	PERCENT CROSSOVERS IN TOTAL DISTANCE	TOTAL NUMBER COUNTED	NUMBER OF CROSSOVERS (ALL SINGLES) IN:				
			Region 1 T^d–b	Region 2 b–p_r	Region 3 p_r–c	Region 4 c–s_p	Total distance T^d–s_p
Treated series, "T"	1.0± .22	901	1	5	0	3	9
Control series, "C"	0.15± .06	2060	0	2	1	0	3
Difference of percents	0.85± .23						
Difference ÷probable error	3.7						

crossovers are necessarily very small throughout, the difference between the total percents (map lengths) is significant, and it is decidedly probable, as well, that the percent of crossovers has been increased both in the center and also in the distal regions. If this is the case, it would seem that crossing-over frequency in the distal regions is more readily affected by X rays when genes that interfere with crossing over are present. In the central region X rays affect crossing over readily both in the presence or in the absence of such genes.

As to the degree of the effect produced, the quotient of treated total map length divided by control is 6.9, but this quotient has little meaning for the making of comparisons with the quotients obtained in the other experiments, since the absolute numbers of crossovers obtained here were so

small that the probable error, ±3.1, of the above quotient, is very large in proportion to its own value. Certainly, however, the total length is affected more here, proportionately, than in the other experiments, since in them the bulk of the length was made up of the unaffected distal regions. There is no question of possible effects of change in double-crossover frequency upon the total length in the present experiment, as no double crossovers occurred either in treated or in control sets.

THE STATISTICAL TREATMENT

In calculating the probable errors, the customary procedure was followed, of regarding each sample as a random one, aside from possible differences due to the controlled element of the treatment (X rays). Any deviations greater than would commonly be found between random samples were, therefore, held to indicate an effect due to treatment. Such is the method which has been followed by MAVOR and by most other geneticists in handling their data.

The fact should not be lost sight of, however, that there may possibly be other, uncontrolled sources of difference between the results in "treated" and "control" series, such as differing composition with regard to "invisible" genes, or with regard to unnoticed or uncontrollable environic conditions to which the parents or batches of offspring may at some time have been subjected. As the parents are divided at random between treated and control series, and no distinction is made in the method of handling of the two series of cultures, such differences would, of course, have an equal chance of existing amongst the cultures of either treated or control series alone, but their random distribution among the members of the two series would add another source of deviation between the two final results, in addition to the effect of the treatment, and to the random sampling of the germ cells which the final counts of each culture represent. GOWEN's (1919) statistical studies on crossing over have shown that the frequency of this event is often so variable, from culture to culture, that we must assume the comparatively frequent occurrence here of "invisible" influences like those mentioned.

Fortunately, the effect of such influences may be gauged in any experiment in which a number of cultures (that is, sets of samples) have been counted in both control and treated series. If such influences are not at work, the cultures in either series, separately, will not differ from each other more than random sets of samples should,—a matter not difficult to determine by comparing the standard deviation of the values in the cultures with the standard error to be expected of sets of random samples

of a size equal to the harmonic mean size of the actual cultures. In that case, then, the ordinary formulae for random sampling may be used in determining the "errors" of control and treated series, and of their differences and quotients, and departures sufficiently exceeding these "errors" may be taken as proving an effect produced by the treatment. If, however, the cultures of either treated or control series prove to differ more amongst themselves than random sampling would reasonably allow, a different method must be followed. In this event, the standard deviation (or "probable deviation") which these cultures are observed to show amongst themselves (in regard to the character considered, such as crossover frequency) must be divided by the square root of the number of cultures forming the series. The resulting value must be used as the standard, or "probable," deviation of the value of this character for the series as a whole (treated or control series, as the case may be), and any theoretical "probable error," calculated on the assumption of purely random samples, will not apply. The value obtained as above is, however, itself subject to considerable (though calculable) inaccuracy, unless the number of cultures in the series is large.

In the case of the present data a considerable number of comparisons were made between the standard deviations shown amongst the cultures of a given series, in regard to particular crossover frequencies, and the standard deviations, or "standard errors," to be expected if these cultures really represented random samples drawn from the same material. The correspondence was close throughout, the differences between observed and calculated standard deviations being within the limits of error to be allowed for the calculated standard deviations themselves. It was thus evident that the use of the ordinary formulae of random sampling would here be permissible for determining the significance of the results, and it was also apparent that since the data from a given series were in this sense homogeneous, they might all be added together, for presentation in condensed form, instead of being shown by individual tabulation of the results concerning all 64 classes of each bottle. Finally, a pragmatic test of the significance of the data for determining the effects of the agent under control (X rays), as contrasted with uncontrolled influences, was forthcoming, when the results of the different experiments were compared and found mutually consistent, the results on the two chromosomes paralleling each other in a way that would be most remarkable if due to unregulated agents.

Another customary, but dubious, statistical practice of geneticists, which was followed here, was the calculation of the probable errors by a formula

which really gave the deviations to which other observed values would have been subject, if the given observed value were itself precisely equal to the true value in the material as a whole. As a matter of fact, however, the true value may be either smaller or larger than that observed, and what is really wanted is the probable deviation that observed values would show, firstly, on the assumption of the largest true value that might "reasonably" have been expected to give the value originally observed, and, secondly, on the assumption of the smallest "reasonably possible" true value. As the larger extreme hypothetical true value would have a somewhat larger error than a true value equal to that observed, and the smaller extreme hypothetical true value a smaller error, the figures that are really desired differ somewhat from the probable errors as ordinarily found. The discrepancy is more pronounced when the values considered are themselves small in proportion to their probable errors. Serious miscalculations are seldom caused in this way, however, and rigorous methods for determining the more strictly accurate values do not seem to have been well worked out. It was not considered worth while, in the present work, to go through the laborious and sometimes doubtful calculations that the use of approximation methods for this purpose would have entailed. This slight source of inaccuracy, then, which is common to nearly all statistical work, applies throughout the preceding and the following calculations, in the case of all probable errors employed,—including those for crossover frequencies, map lengths, differences and quotients.

The value termed "probable error," as here used, is indirectly determined and approximate, as the value first calculated in each case is the "standard error," which is multiplied by .6745 to obtain the supposed "probable error." Standard error, unmodified, might be a better measure of deviations, but "probable error" has been employed in the present tables because it is more familiar to the average biological reader, who is accustomed to seeing it appended to an observed value, after a $\pm$ sign, without any further notation being necessary.

The formula used to find the standard error of the crossover frequency (E_p) was the familiar one $E_p = \sqrt{\frac{p(1-p)}{n}}$, where p = proportion of cross overs (observed) and n = total number of individuals in the count. For the standard error of the map length (E_m) a new formula had to be worked out, the derivation of which is given in a paper which will appear in the November issue of GENETICS; this formula is $E_m = \sqrt{\frac{m(1-m)+2\cdot1d+3\cdot2t+4\cdot3q+}{n}}$,

where m represents the total map length (in proportions, i.e., in "chromosome units" divided by 100), d = the proportion of double crossovers, t the proportion of triples, q of quadruples, etc.

The standard error of coincidence (E_c) also presented a problem not hitherto solved. The solution of this, also explained in the parallel paper, is as follows:

$E_c = c\sqrt{\frac{1-c(a_s+b_s+ab)}{d\,n}}$, where c is the coincidence, a and b are the proportions of (all) crossovers in the first and second regions considered, respectively, and a_s and b_s the proportions of single crossovers in each of these regions (or at least crossovers not involving double crossing over occurring in these two regions simultaneously). As explained in the section on "partial coincidence," n may, if non-adjacent regions are considered, be limited to the number of those individuals not having any crossing over in an intermediate region; in that case a, b, d, a_s and b_s are correspondingly limited, and are calculated on the basis of the limited n; the formula still holds, however.

For the standard error of a difference (E_d) the common formula, $E_d = \sqrt{\sigma_1^2+\sigma_2^2}$, was used, where σ_1 and σ_2 are the standard deviations of the two quantities whose difference is being considered. For the standard error of a quotient (E_Q) the formula $E_Q = Q\sqrt{\left(\frac{\sigma_1}{n_1}\right)^2+\left(\frac{\sigma_2}{n_2}\right)^2}$ was employed, Q being the quotient itself, n_1 the value forming the numerator and n_2 that forming the denominator of the quotient, and σ_1 and σ_2 being the respective standard deviations of the latter. Like the formula for the difference, this formula only holds provided the two values entering into it are uncorrelated, but this must of course be true in the present problems.

For some reason, quotients seem seldom used in genetic statistics, and their probable or standard errors are very rarely seen, comparisons being made almost exclusively by means of differences. There are many problems, however, the solution of which requires the use of quotients and their errors. In the present work, the quotients of treated over control, and their errors, are necessary for the main purpose,—a comparison of the intensity of the effect of treatment in the different regions. The corresponding differences between treated and control in the different regions cannot be used for this comparison, since the size of the difference obviously depends not only upon the intensity of the effect, but also upon the size of the region considered. We may now make an examination of these quotients, in a final analysis of the results of the

TABLE 8

Differences between effects of X rays on "susceptible" and other regions. (Effects measured in terms of quotients (T÷C) of treated divided by control frequencies.)

CHROMOSOME III, LIGHTER DOSE, CENTRAL AND OTHER REGIONS			CHROMOSOME II, LIGHTER DOSE, CENTRAL AND OTHER REGIONS			CHROMOSOME III, HEAVIER DOSE, CENTRAL AND OTHER REGIONS			CHROMOSOME III, HEAVIER DOSE, SUBCENTRAL AND OTHER REGIONS		
Regions involved	Difference between quotients	Diff. ÷P.E.	Regions involved	Difference between quotients	Diff. ÷P.E.	Regions involved	Difference between quotients	Diff. ÷P.E.	Regions involved	Difference between quotients	Diff. ÷P.E.
3–4	0.53±.23	2.1	2–3	0.33±.13	2.5	3–4	0.2 ±.35	0.57	4–3	−0.2 ±.35	0.57
3–2	0.46±.22	2.1	2–1	0.33±.13	2.5	3–2	0.88±.30	3.0	4–2	0.68±.20	3.4
3–5	0.47±.23	2.0	2–4	0.37±.125	3.0	3–5	1.05±.30	3.5	4–5	0.85±.20	4.2
3–1	0.49±.22	2.2				3–1	1.11±.29	3.8	4–1	0.91±.20	4.6
3–rest	0.49±.22	2.3	2–rest	0.34±.12	2.8	3–rest	0.81±.31	2.6	4–rest	0.66±.25	2.7

foregoing experiments, and we may use the errors of these quotients to determine the degree of certainty of the final conclusions.

FINAL ANALYSIS

Reference to the next-to-the-last lines of tables 3 and 5 shows that in the central region a significant or probably significant difference between control and treated was produced in each case (chance 1 in 20 in chromosome III, 1 in 200 in chromosome II, for such a difference to be an "error"), and that in none of the other regions were significant differences produced. This by itself, however, does not prove the thesis that the X rays affected the central region without affecting the distal regions, or even that they affected the center more than the distal parts; for the possibility would still remain that the X rays really affected all regions equally but weakly, if the center had chanced to be a plus random variate and the other regions minus random variates of one effect. To eliminate this possibility, and thus prove a differential effect of the rays, it is necessary to show that the intensities of effect, represented by the quotients, for the center, are significantly larger than those for the other sections. Hence, we are now required to obtain the differences between the quotients for the various regions, and examine the significance of these quotient-differences by comparing them with their own "probable errors." The latter are, of course, found by the formula $E_{dQ} = \sqrt{E_{Q1}{}^2 + E_{Q2}{}^2}$.

A list of the differences between the quotient for the center and the quotient for each of the other regions, together with their probable errors and the ratio they form to the latter, in the case of each of the first three experiments, is given in table 8. In the experiment involving chromosome III and the lighter dose, reported in the first column of the table, it is seen that the difference between the quotient for the center and the other quotient in each case hovers about 2.1 times its probable error. Each of these differences by itself is, therefore, not very significant. The differences between the quotient for the center and the mean of the quotients for the rest of the chromosome is given on the last line. The probable error of this mean value is obtained by calculating the square root of the sum of the squares of the probable errors of the individual values, and dividing the result by the number of these individual values (in this case 4). It will be seen that the difference between the quotient for the center and the mean of the other quotients is 2.3 times its probable error; thus, it is slightly more significant than the individual differences, representing a chance of 1 in 9.5 if there were really no difference between the quotients.

Turning now to the parallel experiment on chromosome II, (2nd column) we find that the quotient for the center differs from each of the others by values ranging from 2.5 to 3.0 times the probable error (representing chances of 1 in 14 to 1 in 23 on the assumption of no real positive difference). The difference in quotients between the center and the mean of the rest of the chromosome is 2.8 times its probable error, which represents a chance of 1 in 17 if the true values were equal (or if the quotient for the center were really smaller).

In each of the above experiments, separately, then, the probability is only moderate that the center was affected by X rays more than the distal regions. We may, however, combine the results from the two experiments, asking the question, what is the probability that in both of them together (i.e., in at least one of the two experiments) the X rays affected the middle more than the ends? To answer this question we may take the mean of the differences between the quotients for the center and for the rest of the chromosome (as a whole) in the two experiments; this value is 0.414. Its probable error (obtained like the error of the means in the preceding pages) is 0.133. Hence the mean difference is 3.1 times its own probable error, which represents a chance of 40 to 1 that the mean difference is significant. We may therefore conclude that it is really very probable that X rays have produced a differential effect upon the center as contrasted with the remainder of the chromosome as a whole, in at least one of the experiments with the lighter dose of rays, and, since there are *a priori* reasons for believing that the two chromosomes would be affected similarly (as well as the argument from the present similarity of data) it becomes extremely likely that both chromosomes were affected in this way.

By following an identical method it may be shown that the chance is 33 to 1 that the central region has in both experiments been affected more markedly by the light dose of X rays than the region (p_r-c or p-s_a) just "to the right" of it, and next in proximity to the center. The calculations involving the center and each of the more distal regions, considered individually, give in every case a considerably higher probability than this. Our original conclusion concerning differential action on the center as contrasted with the rest of the chromosome as a whole may accordingly be extended to each of the regions in this remainder of the chromosome, individually.

The experiment on the third chromosome, involving the stronger dose, proved decisively the effect of X rays on both the region including the central point and that next in proximity to the latter, and failed to show an effect on the distal regions. We cannot, however, conclude that there

was really a weaker effect (much less that there was no effect) on the distal regions until we determine whether the differences between their quotients and those for the more central regions are significant. The third column of table 8 shows that the differences between the quotients for the central and for each of the "distal" regions range from 3.0 to 3.8 times their probable errors (according to the distance of the latter regions from the center), giving chances of only 1 in 20 to 1 in over 100 that these differences have no significance. The differences between the quotients for the p-s_s region and the other regions, with their probable errors, are given in the fourth column of the table. In this case the differences are even more significant than those for the central region (owing to the greater accuracy of the count for the larger p-s_s region); they range from 3.4 to 4.6 times their probable errors (chances 1 in 45 to 1 in 500).

TABLE 9

Effects of heavier dose on composite regions of chromosome III.

SECTION	TREATED FREQUENCY	CONTROL FREQUENCY	QUOTIENT (T÷C)	DIFFERENCE BETWEEN QUOTIENTS	DIFFERENCE ÷ PROBABLE ERROR
r_u-s_t	40.8±.87	41.1±1.19	0.99±.036	0.89±.152	5.9
s_t-s_s	16.9±.65	9.0±.62	1.88±.148		
s_s-e	10.8±.52	11.4±.74	0.95±.076	0.93±.166	5.6

The most decisive manner of handling the data in the above experiment is to compare the quotient for the entire central section from s_t to s_s (including s_t-p and p-s_s) with that for the entire left distal section, r_u to s_t (including r_u-h and h-s_t), and with that for the right distal region, s_s-e. The errors of the quotients are worked out as before, but can now be based on the errors of the control and treated values for composite regions; the latter errors are obtained by the use of the formula previously given for the standard error of a "map length." The quotients and their errors, so obtained, together with the results concerning the differences of these quotients, are given in table 9. The more central "composite region," or "section," differs from the distal section by 5.85 times the probable error of this difference and from the right distal region by 5.6 times the error of the latter difference; the first set of quantities gives a chance of less than 1 in 7000 and the second set a chance of less than 1 in 12,000 that the intensity of the X-ray effect is not greater on the more central region. The differential effects in this experiment, then, are the most conclusively

established of all,—which is in accordance with the fact that a stronger dose was here used.

The results of this experiment with a strong dose differ from those obtained with a lighter dose, first, in that they are more pronounced and decisive, and second, in that they indicate an effect of the X rays over a greater length of the more central region. We may now inquire whether the differences between the effects of stronger and weaker doses are themselves "significant," or whether they could result from random sampling.

Considering first the third chromosome, we find that in the case of the most nearly central region, s_t-p, the difference between the quotient for treated over control with the light dose, $1.52 \pm .21$, and the corresponding quotient with the heavy dose, $2.0 \pm .29$, is $0.48 \pm .36$. As this is only 1.3 times its own probable error it has by itself very little significance. In the case, however, of the subcentral region of this chromosome, p-s_s, there is a difference of $0.81 \pm .21$ between the quotient for the light dose, $0.99 \pm .093$, and that for the heavy dose $1.8 \pm .189$. This difference, being 3.85 times its probable error, would occur but once in more than a hundred trials, if there were really no difference between the effects of the two doses. Thus, it is practically certain that the heavier dose affected at least a part of the s_t-s_s section more strongly than did the lighter dose. While it is quite possible that within this section the subcentral region, p-s_s, has its crossing-over frequency more raised by a change from light to heavy dose than does the centralmost region, s_t-p, (relatively to the effect which the light dose itself causes), nevertheless, the errors are much too large to make sure that there is a real difference between the two regions in this respect.

Turning now to the second chromosome, and considering first the centralmost region, b-p_r, we find that for the lighter dose the treated-over-control quotient is $1.4 \pm .12$. For the stronger dosage we may use MAVOR's data, relating to flies from eggs laid 6 to 12 days after treatment with 65 H (averaging together the results from 62.4 H and 68 H). From these data we may compute, by the methods outlined in the present paper, a quotient of $4.07 \pm .315$ for the b-p_r region. The difference between the quotients for the two doses here, then, is $2.67 \pm .34$. This is 7 to 8 times its probable error, and hence unquestionably significant. On the other hand, for the subcentral region, p_r-c, the data of the present paper, involving the lighter dose, give a quotient of $1.08 \pm .053$, and MAVOR's data, for the heavier dose, may be reckoned as giving $1.23 \pm .055$. The difference here is $0.15 \pm .076$. This would represent a chance of 1 in 5.6 on the assumption of no real difference in effects of the two doses, but about the same chance if the

heavier dose really produced as much increase in crossing over, relatively to the effect of the lighter dose, as in the case of *b*-p_r. For the second chromosome, then, we find an undoubtedly stronger effect on the central section as a whole, *b*-*c*, in MAVOR's experiments than in those of the present paper, but we cannot tell whether the two "regions" of this section are affected to the same extent (relatively to the effect caused by the lighter dose) by a change from lighter to heavier dose.

There is no real ground in the above results, then, for assuming a difference between the second and third chromosomes, in regard to the chief seat of effect within the central section, of an increase in dosage, even though the raw data suggest such a possibility, and this assumption would, if anything, seem rather unlikely, in view of the similarity in behavior otherwise shown by these chromosomes, both in the present and in other experiments. Neither can we, in the case of either chromosome, decide from the present data whether the lighter dose of X rays is really more localized in its place of action than the heavier dose. For, although the lighter dose appeared to produce no effect whatever on the subcentral region of either chromosome, the errors were great enough to allow for an effect as large, in proportion to the effect of the same dose on the central-most region, as that found in the case of the heavier dose. All that can be said is that not until the heavier dose was used were the effects on the subcentral regions pronounced enough to be detected under the conditions of the experiment.

In the case of the second chromosome, there is an additional chance for error in comparing the effect of the two doses, inasmuch as (1) MAVOR's work was done on a stock of widely different origin from that used by the present writer, and (2) we do not know whether the individual cultures in MAVOR's experiment really represented random samples, or varied more than random samples from one another, due to environic or genetic causes. The latter suggestion seems to receive a certain amount of support in the fact that when the results obtained by MAVOR with 62.4 H and 68 H are compared, it turns out that the quotient of treated over control for *b*-p_r was higher with the latter dose, but the quotient for p_r-*c* was higher with the former dose.

We may consider, lastly, the question of possible reversal of the effect of X rays on crossing-over frequency, with change of dosage. This was originally suggested by PLOUGH (1924), chiefly in order to reconcile MAVOR's findings of a decrease in crossing-over frequency in chromosome I, and an increase in chromosome II, when similar doses of X rays were applied. PLOUGH's suggestion was that weaker doses of X rays may cause

a decrease and stronger doses an increase of crossing-over frequency, in the same chromosome (or, we may now say, "chromosome region"); that chromosomes (or "regions") may, however, be different in their susceptibility to X-ray influence; and that, therefore, the same dose of rays may cause a decrease in crossing-over frequency in one portion of the chromatin, and an increase in another portion, which is more susceptible to their action.

In the present experiment, even when considered in connection with MAVOR's results on chromosome II, there is no case of a decrease in crossing over with lighter dose, followed by an increase with stronger dose, in any of the regions studied in either second or third chromosome. If such an effect can be produced on the central region or on the one next in nearness to the center, then both these regions must be so very susceptible to X rays that the critical amount, beyond which a decrease of crossing over is not produced, had already been attained even by the lightest dose used. On the other hand, if such a reversal of effect can be produced on the distal regions of chromosomes II and III, then these regions must be so insusceptible that this lighter dose did not have sufficient strength to cause even a decrease of crossing over in them. With the heavier dose, the data did show a slight decrease in the two distalmost regions studied in chromosome III (there being no data available for the effect of the heavier dose on distal regions of chromosome II), but the decrease was only 2.1 and 0.7 times its probable error in the two respective cases. The average decrease in these two regions taken together was 1.55 ($\pm$.75) percent; this difference, being 2.1 times its probable error, would occur once in seven random samples even if the decrease were not real. The result, therefore, by no means proves a decrease in these regions, and if there is a decrease it is much less than that found by MAVOR in chromosome I. Even if taken at its face value, however, there is nothing to show that a still heavier dose would cause an increase of crossing over in these regions.

If the central regions are so very susceptible and the distal regions so very insusceptible to the X rays, it might be expected that in some intermediate region that was studied,—such as *h-s*, or T^{d}-*b*,—there would be an intermediate susceptibility, such that one of the two doses used, at least,—either the lighter or the stronger one,—would cause a noticeable decrease of crossing over. Such an intermediate susceptibility would be especially to be expected in view of the proved susceptibility gradient in the central regions (the centralmost region having been affected significantly more than the adjacent one, by the lighter dose). There is,

however, no sign of such a crossing-over decrease in the "intermediate" regions. Instead, there are doubtful, very slight, increases of crossing over in these regions, especially with the stronger dose. It is difficult to reconcile this result with the idea of a reversible action with increase in dosage, unless we suppose a peculiar curve of susceptibility along the length of the chromosome. The curve, after presumably falling off gradually from a maximal height at the center, would have suddenly to fall off very sharply, at some intermediate distance, to a level very far below that for the center. While such a situation would be by no means impossible, it must be admitted that it would involve rather special and peculiar conditions, and these special requirements of the hypothesis of a reversible effect of the X rays would argue against the probability of that hypothesis.

SUMMARY

1. The comparatively light X-ray dose of 26.8 Holzknecht units causes a significant increase in the amount of crossing over in the central regions of both the second and third chromosomes (the long V-shaped autosomes) of *Drosophila melanogaster*. In the case of both chromosomes the region so affected is (normally) not much over 6 units long; "subcentral" regions adjacent to this, as well as distal regions, have their crossing-over frequency affected little or not at all by the above dose.

2. a. Application of twice as strong a dose of X rays, in the case of the third chromosome, causes the frequency of crossing over to be significantly above that in the controls not only in the central region but also in the "subcentral" region,—in which, moreover, the difference between the results with the lighter and heavier doses is significant likewise. More distal regions, however, have their crossing-over frequency sensibly unaffected (or possibly very slightly decreased) even by the stronger dose.

b. For the second chromosome, the results of the present experiments with the light dose may be compared to MAVOR's results for black-purple-curved, in which a relatively heavy dose was used. Again we find a significantly stronger effect from the heavier dose, and a significant effect upon the "subcentral" region with this dose only, but data are not available to show the effect of the heavier dose upon the distal regions of this chromosome.

3. The above results prove that there exists in both long autosomes a regionally differential susceptibility to X rays, with the maximum susceptibility in the morphologically differentiated portion at and near the bend of the V. The effect of the X rays is not confined to a single central point however. These intrachromosomal differences in susceptibility are

very great, being comparable in size to the interchromosomal differences found by MAVOR between certain regions in the first and second chromosomes.

4. In view of these conclusions, it will be seen that adequate comparisons of the effects on different chromosomes can be made only after various sections of them, representative of the different kinds of regions comprising them, have been studied. The second and third chromosomes, when studied in this manner, prove to be very similar in their reactions.

5. No evidence has appeared in these experiments for the idea that the X-ray effects may be reversed in sign with change in dosage,—that is, that weaker doses may cause a decrease, and stronger doses an increase of crossing-over frequency, in a given section of chromatin. It is shown that if the idea is correct, adjacent regions in an autosome must differ very sharply in susceptibility.

6. Coincidence of crossing over, so far as it could be studied in these experiments, was affected little, if at all, in either the second or the third chromosomes, by the doses of X rays here used. This result applies to coincidences below 1.0 occurring in distal regions, and coincidences about 1.0, involving either distal or central regions, or both, but central regions short enough and close enough together to give coincidences below 1.0 were not available for study.

7. Even when the crossing-over-inhibiting genes carried in the chromosomes containing "curly" are present in one of the second chromosomes, application of X rays causes an increase of crossing-over frequency. This increase is again noticeable in the central region, but it probably occurs also in the distal regions in this case.

8. Difficulties involved in obtaining the records,—which required the classification of each of the 10,439 flies counted into one of 32 or 64 possible combinations,—were reduced by means of a special method of tabulation, presented in the text, which can be used to advantage in the case of all complex linkage counts.

9. In the statistical analysis of the data, new methods and formulae are employed, which are also applicable to other genetical work.

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